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PREFACE

IT has given me great pleasure to accept the suggestion of the Editor of Harper's Library of Living Thought, that I should treat in a volume of this series some phases in the life processes of plants.

There is scarcely any other question in the biology of plants of greater interest than that of the general chemistry of the cell, viz. of the living protoplasm, which has been so successfully worked at by the biochemists of our time. Not only very important results, but also most suggestive hypotheses, render this chapter of plant Physiology more attractive than any other. The molecular structure of living protoplasm, as well as organic synthesis in cells and the hitherto inexplicable phenomena of endosmosis in the cell, have been rapidly placed in the foreground of modern scientific problems and now range among the great questions of biology to solve which is a well-grounded hope.

So I could not resist the temptation to give a short review of this territory of Biology which is so full of suggestions and attractions. I was, however,

CHEMICAL PHENOMENA IN LIFE

CHAPTER I

BIOLOGY AND CHEMISTRY

THE establishing of the close connection of the biological and the chemical methods of investigation, so familiar in our days to all who are interested in science, was by no means an easy achievement. On the contrary, this was one of the most important and most difficult steps taken in the glorious era of the great French Encyclopædists and Philosophers. Chemistry aims at showing the diversity of matter. It tries to separate and to select, to outline the general laws of proportion in quantity and weight in matter, and it does not appeal directly to our senses. It is only experiments that step by step unveil the clouded path of the investigator and lead him up to the heights from whence he has a clear and far-reaching view over the silent fields of Nature.

Chemical and physical experiments are said to show the laws of Nature. But what do we call

"*Laws*" in Chemistry and Physics? If the conditions of a certain kind of experiment are kept exactly the same, the experiment must invariably lead to the same result. Thus the same result is shown, however often a physical or chemical phenomenon of a certain kind is repeated in Nature itself or by the hand of the experimenting scientist. Single results, as they are produced by arbitrary human action, vary. In a great number of them we may already distinguish a considerable number of average values. Suppose this action is repeated infinitely often, mathematics teach us that we may consider the average result as the true and final value, and we may believe this an equivalent of a *Law of Nature.* We see, therefore, that *Law* in Chemistry and Physics is the expression for the probability of the result when a process repeats itself infinitely often. Thus a phenomenon in Nature, such as the free falling of bodies or the chemical reaction between sodium chloride and nitrate of silver, may with the greatest certainty be expected to take in every case the same course which we have observed even upon only one occasion. Chance and probability are there excluded, and the full certainty of a *Law of Nature* is given. Chemistry in consequence may apply the means of mathematical calculation to the course, and the final results of chemical change in matter. It belongs, as we say, to the *Exact Sciences.*

BIOLOGY AND CHEMISTRY

Biology presents in every line a striking contrast to Chemistry. It does not need experiments to such an extent as Chemistry does. Chemical objects lie unchanged before us, their qualities unaltered, unless we disturb them by experiment. Animated Nature works upon our senses in the most striking manner. In animals and plants gay and bright colours delight our eyes. How much too do we not feel attracted by the different forms of movement in living beings? In the childhood of the civilisation of mankind, as well as in that of the individual, Life and Motion, without any visible external agency, are nearly identical conceptions. The variability of phenomena in animated Nature which are accessible to mere observation without experiments is so great, so infinitely great, that the method of experiment in Biology seemed to be entirely unnecessary to all great naturalists up to the eighteenth century. Much more attention was given to the comparison of the different phenomena of life. This method is what we in our days call *Comparative Biology*. This branch of Biology is particularly occupied with the study of the form and the structure of organisms, that is, *Morphology* and its annexes, *Embryology*, *Anatomy*, and *Histology*.

The more we feel the importance and preponderance of Morphology and of comparative investigation in Biology, the more we must in-

cline to the highest admiration for the genius who first applied chemical and physical methods to Biology. *Stephen Hales' Statical Essays* (1727) are the memorial of the entrance of Physiology into the ranks of the Exact Sciences. These *Essays* contain the first application of physical laws to biological problems. The pressure of blood in the arteries and the pressure of sap in the vessels of plants were henceforth facts expressed in exact mathematical values. In studying *Hales' Statical Essays* we may most strikingly feel the splendid progress in Biology which lies in the application to the ever-changing living organism of methods hitherto only applied to inanimate matter. Experimental Biology entirely abstracts from the qualities which to the naive eye of the observer are characteristics of life. It enters the territory of its investigation from the highest philosophical point of view, that of the probable connection of living and non-living matter.

Thus was built the bridge between Exact Science and Biology. At present we may consider *Experimental Biology* an Exact Science as well as Physics and Chemistry. All employ the same methods, and their end is the same, viz. to lead by means of mathematical conclusions to general results which enable us to explain a greater complex of facts starting from a limited number of experimental results. I would prefer to speak of *Experimental Biology* rather than of *Physiology*, as is

usually done. The very experiment is what is characteristic of the physiologist's method, in the same way as comparison is the chief characteristic of Morphology or Comparative Biology.

We shall not be surprised to first find physical methods in predominance upon the field of Experimental Biology. This was in the age of Newton. Some decades later the work of *Lavoisier* in France, of *Cavendish, Priestley,* and *Ingenhousz* in England, and of *Scheele* in Sweden brought the dawn of scientific Chemistry. It was not a mere chance that the discovery of oxygen was closely connected with the important discovery of the fact that living green plants produce in bright sunlight a considerable amount of the newly discovered gaseous element. We henceforth see Chemistry and Physiology growing as sister-sciences, and no era of Plant-Physiology was richer in important discoveries than that of the foundation of modern chemistry inaugurated by the great *Lavoisier.* At the same time that Chemistry was born, *Biochemistry,* or the knowledge of Chemical Phenomena in Life, came into being.

Every extraordinary advance in Science was accompanied by a revival of materialistic philosophy. The age of *Newton, Lavoisier, D'Alembert,* and *Maupertuis* was the mother of *La Mettrie's* work *L'Homme Machine.* A century and a half before our days imaginative minds even thought of a chemical synthesis of living cells. When

Goethe's poetical genius created Wagner, Faust's famulus, mysteriously mixing hundreds of substances in his retort upon the chemical hearth,

"denn auf Mischung kommt es an,"

it was the reflection upon the great poet of myriads of scientific phantasms of that time, as to whether it were not within the reach of possibility to compound Life itself from the elements which Chemistry had shown to be the pillars of the Universe, and which were contained in every animate and inanimate part of the visible world.

Again, further, the renaissance of Materialism in the last century was the consequence of the marvellous progress of Exact Science, which even showed us the elementary structure of planets and fixed stars, and taught us to construct in the laboratory the vital compounds of animals and plants, such as sugar, fat, and protein bodies, from their very elements.

Here I need not give an extensive sketch of the Natural Philosophy of our time in its relation to Biology, and especially to Physiology. Only a few remarks on the importance of experimental physical and chemical methods in Biology may be added. The enormous advance of our chemical and physical knowledge of the *life process* may easily lead to too far-reaching opinions on the unique significance of these methods. Can *Life* be explained by Physics and Chemistry? Are our

methods in Biophysics and Biochemistry sufficient to disclose the secrets of living cells and to unveil the arcanum of Nature ?

Undoubtedly nearly all the exact physiological knowledge that we possess is based on physical and chemical methods. Every year we are confronted with new and surprising facts in the Physics and Chemistry of animate Nature entirely parallel to facts in the Physics and Chemistry of inanimate Nature. But my conviction is that nevertheless Physiology cannot be really identical with the Chemistry and Physics of living organisms. If we consider the explanation of the fundamental problems of Life to be the aim of Physiology, Physics and Chemistry will presumably not be able to fulfil this great task for themselves alone. It must, however, be conceded that it becomes more and more improbable that Life develops forces which are unknown in inanimate Nature. *Life force* was said to produce the host of peculiar substances which in Nature occur only in living organisms, and are never produced by non-living bodies. These substances were called *organic substances*. The part of Chemistry which deals with organic compounds is even nowadays known as *Organic Chemistry*. The great Chemists of France were the first to show that organic compounds are for the greater part compounds of carbon. The abundance of carbon compounds in the animal and plant world, the scarcity of such

compounds in non-living matter, form a striking contrast. We are, then, not surprised to see that at the beginning of the last century the view was generally adopted that carbon compounds can only be formed by synthesis in the living cell. To be complete it must be mentioned that still in the eighteenth century even the mineral salts in plants were said to be formed in the plant cell by the Life Process. Saussure, in 1804, was the first biologist who proved unquestionably that all mineral salts are taken up into the plant from their watery solution in the soil, and that none are formed in the plant itself.

In 1828 the question of carbon compounds in living organisms was solved by the discovery of the German chemist Woehler, that urea can be artificially prepared in the laboratory from ammonium cyanate. The deep impression produced upon the scientific world by this important synthesis may be gathered from the opinion expressed by Dumas in 1836. The eminent chemist stated that no sharp line of distinction could be drawn between Inorganic and Organic Chemistry. In plants and animals must rather dwell a peculiar power of synthesis which it was henceforth the task of Organic Chemistry to imitate. The glorious range of organic syntheses during the last century is still fresh in our recollection. Nearly all the important animal and vegetable substances are at present accessible to artificial

synthesis from their very elements. Even protein matter seems to have lost its mysteries since we learned from Emil Fischer's work that amino-acids can be combined in the same way as they occur in protein. Compounds of amino-acids can be obtained which show all the main reactions of protein substances. Emil Fischer, of Berlin, was the same chemist who in 1886 discovered how to prepare grape sugar from glycerin. A considerable number of plant alkaloids have been also artificially prepared in the course of the last five decades. The most important colouring matters of plants, for instance, alizarin and indigotin, are no longer extracted from plants for technical purposes, but are accessible from the products of coal-tar. We see, then, that animal and plant substances are by no means peculiar to the realm of organic nature. They are compounded within the living cell and without it by the same chemical laws. Our task in experimental Biology can only be this, to explore the material in the living cell which carries out the chemical changes in substances, and to control the reactions which take place in Life.

The following chapters try to show what success has been attained in the endeavours of Science in the bordering territories of Chemistry and Biology.

CHAPTER II

PROTOPLASM AND ITS CHEMICAL PROPERTIES

DURING its life and in the course of its evolution, the form of the body and its organs is subjected to a continuous series of changes. But at the same pace the organism of the individual undergoes chemical changes. Its general composition is changed. Chemical analysis shows new substances formed, which at an earlier age were not yet present, whereas some substances have disappeared. This is the parallelism of morphological and chemical change in the life of the individual.

Chemical investigation, however, to a certain extent teaches considerably more than Morphology does. We shall prove this in our discussion of chemical reactions in living matter.

Chemical changes in living substance continue without interruption as long as active life prevails. So the chemist has to face great difficulties when examining living matter. From his occupation with inorganic matter he will be accustomed to see that no change takes place in

the matter under investigation unless an experiment be made.

We stand before the question as to what may be made responsible for this continuous change of form and of chemical properties.

Inspection with the naked eye could not have brought any solution of this question. Nor was chemical analysis able to contribute facts of importance. Only to the microscopical investigation of the cells do we owe our knowledge of the organs of life. And here again animal cells have proved to be much less accessible for searching analysis than the cells of plants. It was in 1840 that Hugo von Mohl, of Tübingen, drew attention to the important fact that plant cells have the qualifications of life only as long as they contain a slimy layer along the cell wall, which layer was at first called the *Primordial Utricle*. The thorough examination of anatomical facts led Mohl and Schleiden to the conviction that all the organs of the cell originate in this slimy matter. Consequently the mucous layer was called *Protoplasm*.

In the following decades it was fully established that the presence of life is extremely closely connected with the presence of active protoplasm. The physiologists Bruecke and Kuehne may be called the originators of the view now universally adopted that Protoplasm is the Living Substance in animals and plants. The general and fundamental properties of protoplasm in both are the

same. But it was the merit of the well-known botanist Ferdinand Cohn, of Breslau, that he was the first to declare, in 1850, the identity of the protoplasm in plant cells and of the so-called Sarcode in animal cells.

The Chemistry of Life may henceforth be called the Chemistry of Protoplasm. This is our territory when we study Chemical Phenomena in Life.

The first work the chemist does when beginning his examination of a substance, is to describe its properties before they have been changed by any reaction. We have also to specify the chemical qualities of the substratum of life before we enter upon the effects of reactions between protoplasm and other substances brought into contact with it.

What is protoplasm chemically so called? Is it to be considered as a substance peculiar to living organisms and responsible for all the unique phenomena by which life is characterised? Or is protoplasm a combination of different substances peculiarly composed? Or, finally, is there any unknown structure in the mucous matter which we call protoplasm, and should we not prefer to speak, rather than of a substance or of a combination of substances, of a minutely structured organ when we deal with protoplasm?

Morphology, however, and comparison with other details of cell structure strongly uphold the theory that protoplasm is an intricately constructed organ of the cell. It does not matter

that even the powerful microscopes which the advanced technical perfection of our time has produced, cannot show any more minute morphological details in protoplasm than some very small dark granules or scarcely visible drops of liquid, spoken of as *Microsomes*. But the exact and extremely regular development in the evolution of the cell organs, as well as the undoubted co-operation of protoplasm and the nucleus in cell cleavage and in fecundation, is the strongest affirmation of the organ-theory of protoplasm. In consequence of these facts, we prefer to speak of *Cytoplasm* instead of protoplasm, when we characterise the living substance of the cell, surrounding the nucleus.

Experiment, too, seems to establish such a theory very readily. When animal or plant tissue is minutely pounded in a mortar, the pulpy mass which we finally obtain is far from being an organ, or from containing living cells. It is as little a living thing as a watch remains a watch after having been ground down to powder. Notwithstanding this, the component substances must have remained in either case. It is clear that protoplasm is as little identical with its component substances, for instance, protein bodies, carbohydrates, etc., as pulverised gold, steel, and rubies are identical with the mechanism of a watch. This consideration must lead us to the conclusion that protoplasm is not a

mere orderless homogeneous combination of different substances or a peculiar substance in itself. On the contrary, it renders it very probable that structural characteristics play a most important part in living protoplasm, perhaps form the essential trait in the organ of cell-life. Experimental Biochemistry of our days, however, has been able to show that the characteristics of living protoplasm are not all destroyed at once, when a living organ is ground to a pulp. If care is taken to ward off the effects of microbes which rapidly develop in the remains of the tissues, by adding some toluol or chloroform, a series of reactions which are quite peculiar to life can be still observed in the disorganised pulpy masses. This method of preserving organs which have been minutely ground down is much employed in modern physiology. We call it *Autolysis*. It is possible to prove that autolytic mixtures show the same chemical processes as we find in the digestion of food, in respiration and even in excretion. Therefore we cannot concede that protoplasm is at once destroyed when it is ground down as minutely as possible. The death of protoplasm is no sudden process. The reactions of life cease slowly and successively one after the other.

Theories which maintain that protoplasm is merely effective in life through its structure are generally classified as the *Engine-Theories of Life*. We see that such theories are right essentially,

but that they do not exhaust their subject. They leave unexplained all the phenomena of life which continue in autolytic mixtures. All the theories which lay stress upon the peculiar chemical nature of protoplasm can be called the *Stuff-Theories of Life.* Such a theory was that which was kept in mind when Biology first began the investigation of protoplasm. In consequence of this view analyses were desirable. The analysis of protoplasm should be as correct and complete as possible, in order to show of what kind of substance the substratum of life consists. The difficulty was to collect a sufficient quantity of pure protoplasm for analytical purposes. Reinke and Rodewald in 1880 tried to solve this important question by an extensive analysis of the mucous plasmodium of Fuligo varians. This organism consists of a yellow slimy matter, exactly comparable to the cell-protoplasm of other plants. The result of this famous analysis was to show that protoplasm consists of different organic and inorganic compounds. The greater number of the organic protoplasmatic substances, however, were found to belong to protein matter, *sensu lato.* About $\frac{1}{2}$ or $\frac{2}{3}$ of the dry substance of protoplasm can be considered to be protein bodies. Of the remainder about half were found to be fatty bodies, sugar, and carbohydrates. The other part contained different organic acids, of which amino-acids may particularly be mentioned,

different organic bases, finally mineral salts of potassium, magnesium, and calcium.

Reinke and Rodewald drew from their different experimental work the conclusion that protoplasm could not be considered to be a specific organic substance. It was rather a complex of various organic and inorganic substances, none of which was new to chemistry. In consequence of these experiments the two German biologists inclined to the opinion that it was not chemical and substantial properties which essentially characterised protoplasm, but mainly the structure of the protoplasmatic masses in living cells.

The impression made by this experimental work upon biologists, both botanists and zoologists, was so great that for a long series of years the *Engine- or Structure-Theory* of protoplasm was exclusively the prevailing one. The opinion of Oscar Loew and some other eminent physiologists that protoplasm must nevertheless contain some peculiar matter which is characteristic of life was scarcely taken up by any textbook authors or University teachers.

The last decade, however, seems to have prepared an alteration in the course of the biology of protoplasm. As I have already mentioned, chemical methods clearly show that in the pulp prepared by grinding down living organs in a mortar some vital phenomena continue for a longer time. Therefore not all the Chemical

Life is destroyed, even if cell-structure is as completely as possible annihilated. Consequently some substances must exist in protoplasm which are directly responsible for the life-processes, which do not cease with the destruction of the cell. And these substances *are* characteristic of *living* protoplasm. For when the cell-pulp is heated to the temperature of boiling water these chemical processes cannot be any longer observed. The remainder of the cells may then be considered as definitely dead.

So we must come to the conclusion that, in spite of the ingenious experiments and arguments of Reinke and Rodewald, the comparison between protoplasm and mechanical structure is not quite an exact one. No mechanism is known which would not be destroyed by minutely pounding it, but which is destroyed by boiling water. And, on the other hand, chemical alterations are quite usually caused by a raised temperature, but scarcely in any case by simply grinding down the material. When we see that the substances in living protoplasm are so easily destroyed by heat, we are not surprised that the analysis of protoplasm by Reinke and Rodewald could not detect such constituent parts of living matter. At present, however, it would be possible to carry out exact analytical studies on protoplasm with highly developed methods and with much more success. Nevertheless, the literature of the last

years does not contain more than a few reports about analytical work on protoplasm. The great difficulty in such investigations is to procure a sufficient quantity of suitable material.

Nevertheless, we possess valuable papers on the chemistry of protoplasm from special research work done on animal and plant material. There are results which clearly show the difficulties met with in preparing the protoplasm-proteins without any chemical change during the process of separating them. There is no doubt that protoplasm contains highly complex proteins which are very easily split up into more primitive protein substances, even by treating them with very dilute alkaline or acid solution, or even by keeping them in a watery solution for a couple of hours at ordinary laboratory temperature. Reinke's opinion was that one of the protein bodies of his preparation, the so-called Plastine, was the chief constituent of protoplasm. Later, Etard was fortunate enough to isolate complex protoplasm-proteids of highly variable character. The French chemist proposed to name these compounds *Protoplasmids*. By more advanced methods of quickly drying the cell protoplasm without applying too high a temperature, zoochemists succeeded in preparing a series of such *Organ-Proteids*. We cannot but hope that the biochemistry of protoplasm will in this way make considerable progress. The successful investigations

on the *Enzymes* marked a very important step towards the discovery of the true chemical nature of protoplasm. A special chapter has to be dedicated to these remarkable substances, the properties of which are eminently characteristic of living matter.

The final result of our discussion is that there are many reasons for maintaining that protoplasm really is of a peculiar chemical constitution, and that it does not merely represent a mechanical structure. But we have to concede that the chemical nature of protoplasm is not founded upon the peculiarities of one particular substance which is characteristic of living protoplasm. There are, we are certain of it, a great number of constituents of protoplasm which form the substratum of cell-life.

CHAPTER III

PROTOPLASM AND COLLOID-CHEMISTRY

WE have been told in the foregoing chapter that protoplasm is a slimy mass containing numerous organic compounds which chiefly belong to the groups of proteins, carbohydrates, and fatty bodies. The substances named here represent for the chemist chemical bodies of certain physical properties which, since the famous investigations of Thomas Graham on *Liquid Diffusion applied to Analysis*, in 1861, are well known as *colloidal properties*. Colloids, the prototype of which is glue, τὸ κόλλα, were characterised by Graham as substances which scarcely or not at all show diffusion through animal membranes, and which cannot possibly be brought into the shape of crystals. Colloids, therefore, form a striking contrast to the common mineral salts which readily show diffusion or *Osmosis* through membranes, and which regularly appear as crystals when the solution is concentrated and evaporated. Graham spoke of this stage as the *Crystalloid Stage*. For him, to use his own words, Colloids and Crystalloids were two worlds of matter, quite distinct and without any transition from one to the other.

COLLOIDS IN PROTOPLASM

It marked an important progress in Biology when the views of Thomas Graham were applied to protoplasm. The manifestly colloidal nature of living protoplasm demonstrated *ad oculos* the significance of studies on colloids for Biology. Protoplasm shows itself as an almost liquid slime of the consistence of a liquid starch-paste or of a strong solution of albumin, and never becomes solid. Graham divided colloids, according to their more liquid or more jelly-like consistence, into *Sols* and *Gels*. There is no doubt that protoplasm has the nature of a sol. While the knowledge of salt solutions was being perfected in the 'seventies and 'eighties of the last century, colloidal solutions or sols were also extensively studied. So it was learned that colloidal sols differ from salt or true solutions in a number of important points. Salt solutions are always electrolytes, colloidal solutions never are. Salt solutions have a lower freezing-point and a higher boiling-point compared with the medium of solution (water). Colloidal solutions do not show any divergence from the two principal points of temperature of the medium of solution. Modern physical chemistry explains the properties of true solutions by the hypothesis that, depending upon dilution and temperature, a larger or smaller number of the dissolved molecules are split up into smaller particles which are identical with Faraday's *Ions*. Colloidal solutions do not conduct electric currents and do not show

any difference in the osmotic pressure theoretically calculated from the number of molecules. So we must believe that colloidal solutions are never electrolytes, but are always molecular solutions.

The depression of the freezing-point in solutions is less in proportion as the molecular weight of the substance dissolved is greater. If colloidal solutions only show a very slight depression, or one which lies beyond the limits of exact observation, the conclusion is evident that colloidal substances have a very considerable molecular weight. It was extremely interesting for physiology to learn that exactly those substances which are most important for life possess a very high molecular weight and consequently very large molecules in comparison with inorganic matter. For example, egg-albumin is said to have the molecular weight of at least 15,000, starch more than 30,000, whilst the molecular weight of hydrogen is 2, of sulphuric acid and of potassium nitrate about 100, and the molecular weight of the heaviest metal salts does not exceed about 300.

Thus we come to the hypothesis that the size of the molecules of dissolved colloids is considerably larger than the size of those of crystalloids. It is of great interest that in living protoplasm such large molecules are characteristic of its chemical structure.

Graham believed that colloids and crystalloids are not connected with each other by substances of intermediate character. They were rather said

to differ very clearly. But now we know that *Natura non facit saltus*, not even in colloid and crystalloid matter. The chemistry of proteins showed that typical colloids, for instance, egg-albumin, are step by step transferred into typically soluble substances when these proteins are split up into the products of digestion by the working of digestive ferments. The first products of decomposition, the proteoses, show the typical colloid properties, only slightly less marked than the original protein. The peptones, the next product of decomposition, are not crystallisable, but are distinctly different from typical colloids. Their molecular weight is certainly less than 1000, and they are distinctly electrolytes. Another example of an intermediate state between colloids and crystalloids is demonstrated in soap solutions. Both peptones and soaps are important and widely spread constituents of cell-plasma. Such substances forming transitions from colloids to crystalloids may be called *Semicolloids*. On the other hand, we have to confess that we cannot draw a sharp line of distinction between liquids containing solid particles suspended and colloidal solutions in which only molecules of a large size can be present. These facts are of the greatest importance for Biology.

The chemists Linder and Picton were able to show how suspensions of the yellow sulphide of arsenic are obtainable in particles of all sizes.

From particles which were too heavy to remain suspended and which sank quickly to the bottom, a continual graduation was observed down to particles which were so small that they passed through paper filters and were not even microscopically visible. Bredig's experiments on platinum dispersed by the electric arc in water clearly demonstrated that metallic platinum may be obtained there in every imaginable size of particles. The coarsest particles form a brown precipitate. The finest of them stain the water dark brown without any trace of turbidity, are not retained by any filter, and no particle is microscopically visible. The liquid has all the properties of a colloidal solution of platinum.

The metal-sols, of which a large number have already been obtained, are of great interest, since we possess a new experimental help for studies of colloids in the so-called *Ultramicroscope*. Tyndall drew attention to the remarkable phenomenon that rays of light remain visible in a liquid only when particles suspended therein reflect the light. When water is carefully freed from any trace of particles of dust, we cannot follow the course of rays of light through the liquid. The water rather appears to us as itself diffusely lighted without showing the stripes of light which are produced by a ray of sunlight or electric light thrown upon a vessel containing water. Colloidal solutions always show Tyndall's Phenomenon. This experiment,

therefore, is very suitable to demonstrate the existence of solid particles in colloidal solutions.

About ten years ago Zsigmondy, in Jena, very ingeniously used the principle of Tyndall's phenomenon to show the single particles themselves in colloid solutions by means of the microscope. Whilst microscopical objects are usually illuminated by rays of light so directed that they are parallel to the axis of the microscope, Zsigmondy's microscope was arranged in such a manner that a very thin and strong ray of electric light was thrown through the microscopical preparation from the side, vertical to the axis of the microscope. Consequently the microscopical field of vision remained dark. The suspended particles, when illuminated from the side, reflect the light and become visible, appearing like small stars on the dark sky. The strong dispersion of light does not permit us to recognise the size and shape of the single particles. But they can be counted exactly. In this way the particles of platinum or of gold-sols were made visible, and even their size could be indirectly determined. An arrangement was even made for studying living cells and protoplasm by means of the ultramicroscope. It was clearly shown that numerous particles in protoplasm are made visible by this method which could not be seen by the ordinary microscope.

Ordinary microscopical observation with the strongest lenses can show particles of about 250 $\mu\mu$

in diameter. We call particles of and above this size *Microns*. The ultramicroscope makes particles visible even down to the size of 6 $\mu\mu$, provided that the power of light applied is strong enough. Such particles are called *Submicrons*. But in solutions of albumin or of starch-paste even the ultramicroscope does not dissolve the cone of light into single particles. Nevertheless, it is highly probable that even in such solutions separate particles exist which are smaller than 6 $\mu\mu$. Such are called *Amicrons*. The presence of amicrons can be shown indirectly, for such corpuscules readily become the nuclei of precipitates. When amicrons are present, precipitation is more easily effected than without them. The size of 6 $\mu\mu$ in diameter is probably the size of the albumin molecules themselves. Thus by means of the ultramicroscope it has been made possible to distinguish the largest molecules of colloidal substances and to demonstrate the reality of existence for the molecules. Submicrons, however, are generally already aggregations of molecules. In such a way we can get at least a glimpse of the molecular structure of colloids, and of protoplasm in particular. Protoplasm, in the same way as colloidal solutions, must generally be considered as a heterogeneous system. Solid particles of different colloidal substances are suspended in a liquid. The particles are of different sizes. Some do not differ in size from

large molecules, some form aggregations of molecules, others consist of small masses of the suspended substance, others finally are but coarse particles, already subjected to the force of gravitation, and, if allowed, quietly deposit. The particles, besides, may be of different physical conditions, either liquid drops or solid bodies.

Colloidal solutions, indeed, show quite a different physical behaviour if the suspended particles vary in size and in physical condition. In the first case it is advisable to divide the colloidal solutions into several groups according to the solid or liquid state of the suspended particles. Colloidal solutions which contain solid particles may be called *Suspensions*, such as contain small suspended drops of liquids may be named *Emulsions*. Instead of drops there may even occur in colloids small bubbles of gas. Then the colloid system more or less resembles froth. It is possible that even in protoplasm small bubbles of gas are included, forming a very fine foam.

According to the size of the suspended particles, all these colloids show well-marked physical differences. When the particles are comparatively large the constitution of the system is as a rule very unstable, and the particles are inclined to deposit. Such suspensions are scarcely to be considered as colloidal systems, but rather as a transition stage to colloids. Protoplasm must to a certain extent have the properties of such a sus-

pension. We must therefore ask what characteristics are found in these suspensions. Such systems have in general the properties of the liquid medium. The specific weight, viscosity, and surface tension do not differ from the value found for the medium, and so it is with regard to the freezing-point, the boiling-point, and the power of conducting electric currents. We may understand this to be due to the comparatively small quantity of the suspended substance in proportion to the quantity of the liquid medium. Such suspension systems do not in any way resemble solutions. Here we may mention the so-called phenomenon of *Cataphoresis* in these suspensions. When an electric current passes through the suspension, the particles migrate to the anode or to the cathode, corresponding to the specific character of the suspension. This phenomenon, which has been thoroughly discussed by physical chemists, has not yet shown itself to be of any great importance for the chemistry of protoplasm.

Whilst suspensions with comparatively large particles can be recognised as suspensions by ordinary microscopical observation, the particles in other colloidal solutions can be discovered only by means of the ultramicroscope. We have mentioned that protoplasm contains ultramicroscopic particles or submicrons, which are not seen but by ultramicroscopic investigation. All these

colloids may be called *Suspension Colloids*. From coarse suspensions to suspension colloids there exist all kinds of intermediate suspensions. The platinum sol and the other metal sols mentioned above belong, according to their action and to their physical properties, to the suspension colloids. They have been of great use in studies on suspension colloids. Quantitative analysis showed that even in suspension colloids the amount of the solid phase is very small in comparison with the quantity of the liquid medium Suspension colloids have very few points of resemblance with solutions. They do not conduct electric currents but to a slight extent, and they do not show alteration from the freezing-point of their liquid medium. Cataphoresis has been quite generally noticed even in suspension colloids. In fact, suspension colloids are nothing else but cases of ultramicroscopic suspension. The only one important difference from coarse suspensions is the great stability of suspension colloids. Platinum sol or the colloid solution of hydroxide of iron or any other suspension colloid may be kept for years without showing any alteration. Since the suspended particles are considerably smaller, we must believe that the surface of contact between the suspended substance and the medium (we speak nowadays of the *Medium of Dispersion*) is much larger in suspension colloids than in coarse suspensions. We may consider this to be the reason for the greater stability

of the former. Of great chemical and biological interest is the effect of small amounts of salts, i.e. electrolytes, on suspension colloids. If we prepare a colloidal suspension of mastic resin in water by mixing one drop of alcoholic mastic solution with a large quantity of water, and add to the milky liquid a trace of mineral salt solution, after a couple of seconds white flakes of deposit appear in the colourless liquid, and the whole resin colloid is precipitated in flakes. We do not doubt, and our opinion is confirmed by the noteworthy experimental work of Hardy, Bredig, and others, that the electric properties of the colloid play the chief part in this flaking-phenomenon. We have to think that the colloid particles are aggregated or agglutinised by electric influence, and form a deposit when they have reached a certain stage of aggregation. Probably the particles charged with positive or negative electricity attract ions of the contrary charge. Since ions have a much stronger electric charge than colloid particles, one ion may attract a number of colloid particles. By this process there must be formed large masses of the colloid, which are no longer able to remain suspended in the liquid, and form flakes which slowly deposit.

All colloid solutions or sols which do not show any separate particles either by means of the ordinary microscope or by the ultramicroscope, are at present united under the name of *Emulsion Colloids*. There is no doubt that just such colloids

are the most important constituents of protoplasm. The physical properties of Emulsion Colloids are very characteristic in comparison with those of the suspension colloids. The optical and electrical methods which are so useful in studying suspension colloids do not show remarkable results in emulsion colloids. The suspended particles are so small that their existence can only indirectly be proved by the Tyndall phenomenon.

The particles in suspension colloids are charged with a certain kind of electricity. The organic colloids and the metals of the group of platinum are charged with negative electricity, the hydroxide sols of iron, aluminium, etc., with positive electricity. The kind of electricity never changes. In consequence of this positive colloids may be precipitated by negatively electric colloids and vice versa, but colloids of the same electric charge are never precipitated by each other. The electric conditions are quite different in emulsion colloids. Cataphoresis can be shown, but working more slowly. On the contrary, a very remarkable characteristic of emulsion colloids is that the kind of electricity with which they are charged can be easily changed. Thus albumin particles can be charged either with positive or with negative electricity. It depends upon the chemical condition of the medium of solution which electricity is accepted by the albumin particles. If the reaction of the medium is alkaline, the particles are

negatively electric, but in acid medium they are charged with positive electricity. Emulsion colloids also show quite a different reaction to small quantities of electrolytes. Emulsion colloids are never precipitated by a small amount of mineral salts. The electric properties of the ions cannot alter the colloid state.

Otherwise emulsion colloids in many respects resemble real solutions. In the first place, the diffusion of emulsion colloids is considerable enough to be measured by means of the usual contrivances for studying diffusion phenomena. Such experiments had already been made by Graham. Later on, Pfeffer carried out experiments on solutions of gum-arabic and glue, to show that distinct osmotic pressure can be observed to be exercised by such colloids. The osmotic pressure, however, is very small as compared with the osmotic effects of sugar solution or of inorganic salts. Even the freezing-point of emulsion colloids is distinctly lower than the freezing-point of the pure medium. Such sols show many transition characteristics to true solutions. The density of sols is distinctly different from the specific gravity of the pure medium. The surface tension of sols also differs regularly from the surface tension of the pure medium. In many cases the surface tension of water is lowered by dissolving colloids in it.

Such characteristics are to be expected in the emulsion colloids of protoplasm. Protoplasm,

therefore, has many of the physical and chemical characteristics of true solutions. On the other hand, properties must be present in protoplasm which are only found in suspensions. We see that such a state of things is very favourable for the action and counteraction of many substances in the narrow territory of the protoplasm of one cell. Water is without doubt the medium of solution in protoplasm. Many substances, chiefly of the groups of protein bodies and carbohydrates, form the mucous emulsion colloid which is the fundamental mass of protoplasm. Protoplasm is practically an albumin sol. We remember that fatty substances are regular constituents of protoplasm. They are not soluble in watery mediums, but they may be brought into the form of colloid solution in water, either only into the stage of suspension colloids, as we can see on shaking oil and water together, or even into the stage of emulsion colloids. The latter can be reached by adding a trace of potassium carbonate to the mixture of oil and water. It is sufficient to shake the mixture for a very short time to form a milky liquid of great stability, which can be filtered without change. The physical properties of such oil emulsions are the properties of emulsion colloids. In protoplasm fats must be present in the form of suspension colloids and of emulsion colloids. Other substances insoluble in water must be present in similar forms.

It may be that the whole mass of protoplasm is not equally rich in these suspensions. As a rule we perceive along the cell wall on the outmost layer of protoplasm a thin protoplasmatic part which does not show any visible particles, and only very few under the ultramicroscope. This layer was named by Pfeffer *Hyaloplasma*. The other parts of protoplasm usually contain great quantities of coarser particles which give a greyish colour to the whole protoplasmatic mass. Pfeffer introduced the name of *Polioplasma* for this part of the cytoplasma.

It is manifest that Hyaloplasm is an important medium to admit substances from outside into the cell as well as to permit the passing out of products of the cell. Hyaloplasm can therefore be considered to be the cell organ for the Endosmosis and Exosmosis of substances, i.e. the osmotic organ of cell protoplasm. Polioplasm, on the other hand, must be the organ to assimilate the substances which enter the cell, to form new constituents of protoplasm, to furnish different forms of physical energy, to continue the process of life and to form the substances which are superfluous for cellplasm and are excretions. Polioplasm is thus the seat of the metabolism of the cell itself. We shall try to show how far our present chemical knowledge may explain the connection of all these functions of living cell protoplasm.

CHAPTER IV

THE OUTER PROTOPLASMATIC MEMBRANE AND ITS CHEMICAL FUNCTIONS

BESIDES the transparent condition and the absence of coarser granules or microsomes hyaloplasm exhibits a series of microscopical peculiarities. It is well known that protoplasm in living plant cells generally shows a streaming movement which is easily recognised either by the movement of the chlorophyll bodies themselves or by that of the microsomes. These bodies are carried along by the streaming protoplasm with considerable velocity. Even the cell nucleus is in some cases carried along by the current of streaming protoplasm. This outer transparent layer is continually at rest, is never made turbid by particles, and never includes drops of liquid, cell sap, which is quite commonly found in the polioplasm of older cells. Perhaps the viscosity of hyaloplasm is greater than that of polioplasm. In any case the boundary lamella of the hyaloplasm must be of tougher consistence, and may be well considered to be a plasmatic membrane or boundary membrane of the living parts of the cell. This plasmatic membrane is the proper organ for

regulating the osmotic change of substances with the outer world. While the cellulose membrane of the cell is only a dead cover of the living contents, the living plasmatic membrane is variable in its condition and is quite different when in its normal living state and when dead. If slices of beet-root are dipped in water, after having the remainder of the cells which were cut through properly washed off, one may keep them in water for any length of time without losing even a trace of the red colouring matter in the living cells. But if chloroform is added to the water and the cells are killed by the narcotic agent, streams of red colour go out from the tissue. The dead protoplasmatic membrane is no longer able to retain the contents of the cell.

In the living cell the decision to take up dissolved substances from the liquid outside the cell lies with the protoplasmatic membrane. Even the well-known fact that the chemical constitution of plants is quite different from that of the soil in which they are growing, proves the elective influence of the protoplasmatic membrane in endosmosis. This elective influence is much better shown by the phenomenon of *Plasmolysis*.

We owe to Hugo de Vries, of Amsterdam, the excellent method here described. It is best to choose cells with red-coloured cell sap for the experiments. Such cells are found on the under surface of many leaves. Corollary petals may also

well serve the purpose, but they are not so easily cut with the razor. When such sections are put into salt solution of sufficient concentration, e.g. potassium nitrate 2 per cent, after some minutes all cells show their protoplasm shrunk away from the cell wall. The cell protoplasm forms a red ball lying free in the cell. When the sections are put back into water, the plasmolysis disappears and the cells regain their normal condition. Plasmolysis is therefore a normal, merely physical phenomenon, not at all a pathological one.

How can plasmolysis be explained? Microscopical inspection immediately convinces us of the fact that the volume of protoplasm is reduced in plasmolysis. It was only possible for this to be brought about by the expulsion of water from the sap vacuole of the protoplast. By loss of water the concentration of the sap is increased, until the osmotic value of the outer solution is greater than the osmotic value of the cell sap. This state being arrived at, equilibrium is regained. We learn from this process that the protoplasmatic membrane cannot be permeable for the salt in solution. If it had been permeable, the equilibrium would have been reached simply by endosmosis into the cell, as long as the concentration inside and outside had not become equivalent. Or osmotic substances would have penetrated the protoplasmatic membrane from the inside of the cell when plasmolysis disappeared in water. Consequently, we may say

that the plasmolytic power of a certain solution proves distinctly that the substance cannot pass through the living protoplasmatic membrane. If the solution does not effect any plasmolysis, we may be sure that the substance enters the cell more or less considerably.

Ernest Overton was the first who thoroughly investigated these interesting problems in 1895. He found that mon-acid alcohols, aldehydes, and ketones, also esters of fatty acids and alkaloids, produce least plasmolysis. As a rule it is impossible to bring about plasmolysis by means of these substances. They enter the cell very easily and pass through the plasmatic membrane without any difficulty. Glycols and amino-compounds cause plasmolysis a little more readily. With glycerin or erythrite it is still easier to bring about plasmolysis. But the sugars and the substances most closely related (for instance, mannite), the amino-acids and the salts of organic acids very readily produce plasmolysis. They cannot pass through the protoplasmatic membrane but with great difficulty. Finally, the salts of inorganic substances very quickly cause plasmolysis, since they very slowly pass the plasmatic membrane, or practically do not pass the boundary of protoplasm. Overton added to his valuable experiments a most ingenious conclusion. He drew attention to the fact that just such substances easily pass through the protoplasmatic

membrane as are soluble in fat. This is the reason why chloroform and ether are so readily taken up by the cell. Overton showed further that the phenomenon of narcosis is principally founded upon the storing of chloroform by the fatty compounds which are most important constituents in the nervous system. Overton's theory was at last confirmed by experiments on aniline dyes. These substances as a rule are soluble only in alcohol or in such organic liquids as dissolve fatty compounds. They are readily taken up by cells. It is easy to prepare from such colouring matters compounds which are soluble in water. This is done by treating them with sulphuric acid. The sulphonic acids thus obtained are substances soluble in water, but insoluble in ether or alcohol. Such solutions cannot enter living cells.

The conclusion finally drawn by Overton from all these facts was this, that protoplasm is enveloped in a thin layer which is either rich in fatty substances or is a thin film of fat or oil, as was the opinion expressed by the German physicist Quincke some years before Overton's work appeared.

There are many facts, indeed, which seem to make such a theory very plausible. Living protoplasm always acts as liquids do in a state of equilibrium. When it enters a state of rest it assumes the shape of a sphere. Such action can be quite distinctly seen in amœba when they are

preparing for the resting state. Plasmolysed protoplasm has the same inclination. We see that protoplasm in rest has the tendency to diminish its surface as far as possible in proportion to its mass or its volume. The spherical surface is the geometrical minimum of surface for a certain volume. From this phenomenon we learn that the force of surface tension must in some way regulate the outlines of living protoplasm. When the living protoplasm of an amœba stretches out its so-called Pseudopodia on one side, and draws in the projecting parts on the other, thus creeping slowly over the moist ground, variations in the surface tension on different parts of the circumference of the cell must take place. The surface tension must increase when new prominences are formed, and surface tension must diminish whenever Pseudopodia are drawn in. But such alterations in surface tension presume certain chemical changes in the boundary layer of the cell, and formation of substances which show different surface tension in comparison with the foregoing state. We learn, further, that such chemical processes must be reversible, to be repeated whenever needed in cell life. In water protoplasm always shows a distinctly lower surface tension to the watery medium than mucous protein substances or carbohydrates. It always rounds to spherical shape when in rest.

THE PROTOPLASMATIC MEMBRANE

We owe to the famous thermodynamic studies by Willard Gibbs, the eminent American scientist, the theoretical basis for the knowledge of the behaviour of different substances in compound systems which possess different surface activity. If these substances have the power of diminishing the surface tension of the medium, they always show the tendency to accumulate on the surface. If there are several such substances, then that substance which most depresses the surface tension, or is most surface-active, is generally accumulated in the surface layer. Upon the basis of Willard Gibbs' theory we may expect in advance that all the protoplasmatic substances which have the strongest power of depressing surface tension, such as fats, must necessarily be collected upon the surface of protoplasm. So Overton's hypothesis is confirmed by several arguments, and we may consider it to mark an important progress in the chemistry of protoplasm. In the course of these investigations it was highly desirable that we should be enabled to measure the surface tension of living protoplasm, and to compare the surface tension of protoplasm with the figures obtained for the surface tension of different substances. The difficulties, however, were great and could not be overcome till lately. The advance sought for came from studies on the toxic effects of alcohols on living cells. Traube, in Berlin, showed that the well-known law of the

poisonous effects of alcohols, generally called *Richardson's Law*, that the higher members of the series of alcohols are more poisonous than the lower ones, was connected with the capillary properties or the surface tension of the alcohols. The German chemist proved that the surface activity of the alcohols increases from one member to the following one in the same series in the ratio 1 : 3. A glance at the results obtained by Overton and others on the poisonous effects of alcohols immediately showed Traube that the toxic effect increases in the same proportion. The law of surface activity and Richardson's Law must therefore be the same. Later on, corresponding facts were found in the class of esters, but exclusively in the members of an homologous series of organic compounds.

When I studied the toxic effects of organic solutions on plant cells I noticed that the exosmosis of substances from the cell vacuole, consequently the death of cells, regularly took place when the surface tension of the solution had reached the same degree. Most plant cells are injured and die when a solution is applied which has the surface tension of about two-thirds relatively to that of water. No alcohol, no ether nor narcotic has been found which did not affect the cell in a solution of such a surface activity. But all substances of the most different chemical character began to injure the cell just when the surface tension had reached the critical point. Since all

alcohols, ethers, ketones, and many other substances obey the same physiological law, we must conclude that all these substances have the same physiological effect upon living protoplasm. If we consider that according to Willard Gibbs' theory a substance of higher surface activity, when brought into contact with protoplasm, must necessarily displace the active substances of the superficial layer, we see that disorganisation of the structure of this layer must be the consequence. We understand that exosmosis must take place. This effect is always exercised whenever the concentration of the substance exceeds the critical degree of surface tension. This degree therefore must be slightly below the real value of protoplasmatic surface tension. Consequently we measure also the surface tension of protoplasm, when we apply alcohol or any other solution of the critical capillarity. Practically we may take the surface tension of common plant cells as equivalent to the surface tension of 11 % ethyl alcohol.

This result forces us to raise the question why the surface tension of protoplasm has just this value and no other. Further experiments on the working of fatty emulsions on living cells showed me that poisonous effects such as are produced by alcohols can be caused even by emulsions of fatty bodies, that is, by colloid solutions. The only condition is that the surface tension should be low

enough to affect the superficial layer of protoplasm. So lecithin or cholesterin emulsions are quite as effective as true surface-active solutions. But emulsions of neutral fats never produce toxic effects. The determination of the amount of surface tension in emulsions of neutral fats as highly concentrated as possible, gave the result that such emulsions regularly depress the surface tension to two-thirds of the value of that of pure water. Since fatty compounds are always present in protoplasm, it does not seem to be by chance that the surface tension of living protoplasm and the surface tension of fat emulsions are practically the same. The conclusion may perhaps therefore be drawn that the superficial layer of protoplasm contains an emulsion of neutral glycerids, such as triolein, linolein, ricinolein, and others.

Overton's and Quincke's theory that the peripheral layer of protoplasm can be compared to an oily film or a very thin layer of fat (Overton thought of lecithin or cholesterin) does not seem to be quite a correct one. The ordinary food of plants consists of watery solutions of substances which are usually not soluble in fat. It is, as I think, more probable that the fat in the plasmatic membrane is present in the form of an emulsion of extreme fineness. The interstitial space between the fat-globules must be filled up with a watery colloid solution, most probably a protein

sol. So the plasmatic membrane would in my opinion consist of two phases. One, the lipoid phase, is given by a fat emulsion, the other, the hydroid phase, by the protein solution which forms the greater part of hyaloplasm.

The *Theory of Osmosis,* or the diffusion of dissolved substances through membranes, has undergone many changes. There was a time when it was generally believed that the diosmosis of a substance depended upon the size of the pores of the membrane and the size of the molecules of the dissolved substance. Diosmosis cannot take place when the pores are too small to let the molecules pass. The membrane was considered to act like a sieve for the molecules. This hypothesis does not explain why fatty substances cannot pass membranes which have taken up water. All signs show rather that solution affinities play the most important part in diosmosis. The membrane is always permeable for a certain substance, when this substance is soluble in the material of the membrane. Nernst demonstrated this view by a clear experiment. Ether is soluble in water as well as in benzene. Benzene is soluble in ether only, and insoluble in water. When a quantity of benzene and a quantity of ether are separated from each other by a layer of water, it is to be expected that the ether will go through the layer of water, but not the benzene. A continuous stream of ether will pass through the water, but no stream

of benzene in the contrary direction. An osmotic pressure must be produced, therefore, in the system on the side of the benzene. When the experiment is carried out animal membrane saturated with water is placed, instead of a layer of liquid water, between the ether and the benzene. The benzene is poured into a glass funnel connected with a glass tube, and the funnel is closed with the saturated membrane. Then the funnel is dipped into a vessel containing ether. After a certain time the liquid rising in the glass tube shows the endosmotic streaming in of ether, subsequently the osmotic pressure.

In the foregoing description the term *Plasmatic Membrane* has often been employed for the superficial layer of hyaloplasm. We have to justify the choice of this expression. Membranes are films of firmer consistence than the material, viz. the liquid upon the surface of which they are formed. So the expression plasmatic membrane implies a firmer consistence for this layer than for the hyaloplasm itself. We know from daily experience that a colloidal solution such as a solution of albumin or starch paste, is inclined to form a thin film on the surface, which has almost the physical condition of a solid substance. Protoplasm, being a colloidal system, will most probably not differ from other colloids in this respect. We notice, indeed, after a lesion of a cell when the cell and its protoplasm have been cut through, that the surface of the

wound is quickly covered with a fine film. This may be seen very distinctly in the wide cell tubes of the marine alga *Caulerpa prolifera*. The film-like excretion protects the protoplasm from any further injury from water oozing in. Consequently the whole hyaloplasm layer in the wounded spot is soon regenerated.

The formation of membranes and of films is, then, a general characteristic of protoplasm and of colloids. This goes so far that it is possible to deprive an albumin solution entirely of its contents of albumin by shaking it. The albumin at once becomes insoluble. We see thus how unstable many colloids are. It has been already mentioned in a former chapter that a minimum of salt solution is sufficient to precipitate suspension colloids. But to bring about the flaking out of emulsion colloids by means of salts, we must add comparatively large quantities of mineral salts. There is no doubt that the effect of salts on emulsion colloids is in many respects allied to the effects of dissolving. Between the particles of the colloid and the salt there must be some solution-affinities which do not exist in suspension colloids. In consequence of this characteristic Perrin has proposed to name the suspension colloids *Lyophobic Colloids*, because there no solution affinities play any part, and to name the emulsion colloids *Lyophil Colloids* from their connection with real solutions. Durable films are formed especially by precipitated

suspension colloids. Such precipitations are not reversible. When, on the other hand, albumin is precipitated by sodium chloride, it is possible to again dissolve the precipitation by diluting it with water. This process is reversible. Generally in albumin all precipitations with the salts of the light alkaline metals and of magnesium are reversible. But they are not reversible when precipitated by copper salts, iron salts, or any other salt of heavy metals. Precipitations with calcium or strontium salts are inclined to be quite insoluble in water. It is noteworthy that the working of the salt depends upon the acid contained in it. Francis Hofmeister, of Strassburg, was the first to show that alkaline metal salts of different acids have a certain graduated effect on colloid solutions. They may be arranged in the following way, beginning with the acid which precipitates most quickly:

Citrate, Tartrate, Sulphate, Acetate, Chloride, Nitrate, Chlorate.

This law became of the greatest importance in the chemistry of colloids. It is not only applicable to the transition of colloid solutions into solid colloids, but even to the chemical and physical states of solid colloids themselves.

Graham named solid colloids *Gels*, the name corresponding to that of *Sols* or liquid colloids. The physical condition of certain gels is very different. Glue itself, when quite dry, forms a

horny mass, hard, inflexible, and brittle. When it is more or less saturated with water, it becomes flexible, viscous, then gelatinous, and in the course of imbibition with water it approaches the liquid state. Many gels have the character of a gelatinous mass. Some, as gum-arabic, finally dissolve entirely. Others, as cherry gum, swell in water to a jelly and never dissolve. Doubtless gels are of great importance in plasmatic structure. They are formed in plasmatic colloids by many influences, such as surface tension, electrolytes, and the mutual precipitating effects of colloids. Wherever protoplasm sols meet precipitating influences, films must be formed, which separate the different parts from each other. Such gel-membranes, on the other hand, play the part of semi-permeable filters. Some substances are soluble in them, and consequently pass through, but other substances being insoluble in the gel substance are retained. There is still another retention of substances in gels which is not a consequence of their insolubility, but, on the contrary, must be traced back to some affinity of the substance retained with the gel colloid. We call this process of retention *Adsorption of Substances.* There is no doubt that adsorption is of the greatest importance for chemical processes in life. We have especially to consider that the resorption of dissolved substances by cell protoplasm from the surrounding liquids must be connected with ad-

sorption in protoplasm colloids. Taking up food by hyaloplasm is consequently as inseparable from adsorption in the colloidal matter of the plasmatic membrane, as from solution in the fatty substances of the superficial layer of protoplasm.

Essentially adsorption cannot be separated from the swelling of gels in water. Many experiments have shown that all influences which further the swelling of gels hinder adsorption and vice versa. Hofmeister's Law was found to be in force even in this group of phenomena. The anions of acids which are most effective in precipitating sols are the same which are most adsorbed.

When adsorption of salts takes place by living cells or by colloids, the electric state of the colloid is very frequently of great influence on the process of adsorption. Most of the organic colloids are, as was shown above, negatively electric. They must consequently act like acid anions, and will in adsorption chiefly attract the bases of the salts. If the salt is in a highly diluted state practically adsorption only of ions can take place. Mainly the cations, viz. the metal ions, are retained by adsorption, while the anions remain to a certain extent unaffected. Hence, of course, must result reactions of acids, without any chemical production of acids. Doubtless such adsorption phenomena are of great interest for physiology. It has for a long time been well known that roots of plants produce the effect of acids upon the soil

and its constituents. It is possible to show this by letting roots grow along polished marble plates. After some weeks the marble surface clearly demonstrates the dissolving effect of growing roots and root-hairs. Delicate traces are everywhere etched in the marble surface, where roots have come into close contact with the plate. I was able to show, in 1894, that carbonic acid is certainly to a great extent responsible for this phenomenon. I made plates of plaster of Paris mixed with different substances, the solubility of which in water saturated with carbonic acid, had been well considered. I discovered that only such compounds are dissolved by the plant roots and their excretion, as were distinctly soluble in carbonic acid. These were phosphate of calcium and strontium, but not aluminium phosphate, which is dissoluble by carbonic acid. Nevertheless, there are other effects of acids in plant roots which cannot possibly be due to carbonic acid, and which have not been explained until lately. Now it is believed to be highly probable that merely the adsorption effect takes part in these phenomena, and no excretion of acids by the roots is to be assumed. If the cations are adsorbed and anions of acids remain reactions of acids must result as well as in real excretion of acids. Now we can understand why acids could not be discovered in the excretion drops of the root-hairs, and why they react quite

neutrally. Most probably even the acid properties of peat and of Humic Acids of the soil can be attributed to colloidal elective adsorption. The negatively electric colloids of the peat moss retain, as Baumann and Gully have lately shown, chiefly the basic ions of the dissolved salts, and this adsorptive election must lead to reactions of acid in the soil extract. It can easily be demonstrated that the citrate and the tartrate are most adsorbed and productive more of the effect of an acid than other salts of the same alkaline metal. I cannot but suppose that the taking up of dissolved salts by living cells is essentially founded upon phenomena of adsorption. This opinion has been confirmed by the chemical analysis of peat moss by Baumann and Gully. It was found that the quantities of the basic mineral constituents of the moss-ash are almost the same as are adsorbed by the plant from the soil. Long ago agricultural chemists had stated that the constitution of the ash of plants which grow upon the same territory may widely differ. This elective assimilation of soil constituents may be now explained to a great extent by the adsorptive qualities of the colloids of the living cells.

In summing up we may say that the superficial layer of cell protoplasm, called hyaloplasm, may be considered to be a film of more solid constitution which we call the plasmatic membrane. This membrane regulates the change of substances

in the metabolism of the cell, the assimilation of food taken up from outside the cell and the excretion of substances from the cell. The plasmatic membrane is not completely permeable for all substances, but has a so-called semi-permeable layer, which permits some substances to pass through and others not. The protoplasmatic membrane is a compound colloid system consisting of an extremely fine fat emulsion suspended in a hydrosol, probably an albuminous colloidal solution. We see, then, that fatty bodies are taken up as well as watery solutions. Concerning the latter, we are able to show how important adsorption phenomena are in assimilating them. The laws of adsorption govern the assimilation of salts from the soil. Even the action of acids can be produced by adsorptive election.

So we may say that a great many phenomena in life once attributed to Life Force, and not to be explained by chemical laws, can in the present stage of science be reduced to the general Laws of Nature.

CHAPTER V

CHEMICAL PHENOMENA IN CYTOPLASM AND NUCLEUS OF LIVING CELLS

THE main body of protoplasm, which is surrounded by the hyalin layer of the superficial cell plasma, generally contains finely-granulated, slimy masses of a yellowish grey hue, whence it is named Polioplasm. The appearance of polioplasm is very different according to the age and the stage of life of the cells. Quite young cells are found equally filled with homogeneous polioplasm. In the midst of this protoplasmatic mass one perceives a spherical body of more solid condition which refracts light strongly: the *Nucleus of the cell.* In the course of growth the polioplasm soon produces drops of liquid contents in greater or smaller number. These drops increase in size, and the polioplasm between them changes into thin lamellæ separating the contiguous cavities. The polioplasma gains the character of foam. The cavities between the meshes of tough colloid mass are generally known as vacuoles. The further development shows the conflux of several neighbour vacuoles to one of larger size. The meshes of

protoplasmatic threads and lamellæ become finer and rarer. Only along the cell wall a thick polioplasmatic layer persists. At last, when the cell has nearly reached the definite size, we see, as a rule, only the polioplasma layer along the cell wall, surrounding one large vacuole which occupies the whole central space of the cell. Even the nucleus, formerly suspended on numerous fine plasmatic threads and lamellæ in the middle of the cell, is now situated in the plasma layer near the wall, forming a protuberance in this layer. The general impression is that the mass of protoplasm does not increase when cells are growing in length and diameter. The nucleus even looks a little smaller in adult cells than in young ones. Further, the protoplasma must take up a considerable quantity of water to form the vacuoles and to fill them with a watery solution of different substances, which solution is known as cell sap. Doubtless the mechanism employed in forming vacuoles is connected with the mechanism of growth. The whole bulk of polioplasma does not swell when cells are growing. The quantity of water in polioplasma itself seems to remain constant during the formation of cell sap in the vacuoles.

It is noteworthy that the polioplasma remains pressed against the cell wall. Loss of water immediately disturbs this normal state. Leaves, when withering, lose their normal elastic and firm condition, and at once their capacity of growth.

Distinctly the same effect is produced by the action of salt solution. A flower stalk or leaf stalk of fleshy consistence put into potassium nitrate of about 2 per cent very soon becomes unelastic, flexible, like a withered plant, and shortens its length by some millimetres in a length of about 10 centimetres. We learn from this phenomenon that the pressing of protoplasm against the cell-wall is due to osmotic forces. Hugo De Vries showed, in 1884, that it is possible to use the suppression of osmotic pressure in cells or of the *Cell Turgor*, as botanists say, by salt solution, for the measurement of the osmotic pressure in normal cells. The procedure is essentially identical with the so-called plasmolysis we have spoken of in a previous chapter. One has to apply solutions of a pure mineral salt of different concentrations. It is usual to take potassium nitrate because it is easily available in quite pure preparations and because the percentage in solutions is nearly identical with the standard gauge in chemical work, the *Gramm Molecule Solution*. Solution of potassium nitrate containing 10·1 gr. in 100 gr. water is only slightly more than 10 per cent concentration, and is a molecular solution, containing one gramm molecule potassium nitrate, 101 gr. in 1 litre of water. If we put sections of plant tissue in different potassium nitrate solutions from 0·05 normal to 0·2 normal strength, we find that the separating of the protoplasm from the walls

begins in solutions of about 0·12 to 0·15 normal strength. This salt concentration gives us a gauge for the amount of turgor. De Vries showed that all salts produce the same result at the same concentration in gramm molecules. We call such solutions which have the same osmotic effect *Isosmotic Solutions.* If we are able to directly measure the osmotic pressure of one isosmotic solution, for instance, of a sugar solution, by an osmometric contrivance, we may transfer this value to the osmotic pressure in the cells. So it was found that the osmotic pressure in cells is equivalent to five and more atmospheres, one atmosphere being equivalent to about 0·3 per cent of saltpetre.

The action of polioplasma on the growth of living cells consequently consists in the production of substances which generate osmotic pressure. We know that only such substances as do not penetrate the protoplasmatic layer are capable of producing osmotic effects. It is very little known what substances having that effect are generally produced by plant cells. It is seemingly highly complex acids related to sugar which participate in generating turgor effects in living cells. Introductory to the process of growth a certain amount of turgor pressure is indispensable. We have to assume that by that pressure protoplasm as well as the cell wall is thinned and first stretched, then new particles of cell wall substance are inserted,

by which process the expansion of the cell wall becomes permanent.

The most striking feature of cell life is the fact that an enormous number of chemical reactions take place within the narrowest space. Most plant cells do not exceed 0·1 to 0·5 millimetres in diameter. Their greatest volume therefore can only be an eighth of a cubic millimetre. Nevertheless, in this minute space we notice in every stage of cell life a considerable number of chemical reactions which are carried on contemporaneously, without one disturbing the other in the slightest degree. How can we explain this striking phenomenon ? In the first place we must state that polioplasm is highly specialised in its different parts. Besides the nucleus, which certainly is the seat of most important vital activities, we find many organs which are to be recognised with the aid of the microscope as distinct protoplasmatic organs, and we already know the functions of many of them. Most plant cells contain clearly differentiated small bodies of different shape which are employed in the service of the assimilation of sugar and carbohydrates. In common plants they are green in colour, and possess the remarkable power of absorbing carbon dioxide, if bright light is admitted, and of forming sugar from the carbon dioxide and water. These are the chlorophyll bodies or *Chloroplasts*. Very little is as yet known about their detailed structure.

In my laboratory it was lately shown that the consistence of chloroplasts is often very soft, very much less solid than the nucleus. They contain a mixture of two kinds of colloids, one of them which swells in water, of hydroid character, the other resembles fats and most probably contains the green colouring matter or Chlorophyll. In life, as we may think, the lipoid phase is distributed as a very fine emulsion through the hydroid phase.

There are some other small bodies which are free from colouring matter, and which form starch from sugar. We call all these protoplasmatic organs which are in the service of carbohydrate metabolism, *Plastids*. As far as we know, they are never formed from other plasmatic parts. They always take their origin from mother plastids by cleavage. In some plant cells there have been found special plasmatic bodies which form fat, but more frequently fat is independently formed in the fundamental plasma substance. We may say the same of the protein substances of protoplasm. It may be, however, that for the formation of all these compounds very small centres or distinct organs exist, which cannot yet be recognised even by means of the highest microscopical power. In any case, the parts where the different chemical changes take place must be separated in some way from each other, to prevent mixing with other substances. In colloid systems, as such separating walls, we find membranes formed of

precipitated solid colloids or gels. From the small size of these separated parts the whole protoplasm must have the appearance of a foam formed by gel walls, inclosing in its meshes colloids of more liquid state. This hypothesis is not without support from experiments. The eminent zoologist Bütschli, of Heidelberg, has shown for many colloids, both inorganic and organic, that they have a foam-like structure which may be in some cases observed through the microscope. Evidently such foam-like structure in protoplasm must facilitate the great variety of chemical processes carried on contemporaneously in the narrow field of a microscopical living cell.

These structures can be transitory as well as permanent. It is very probable that in the course of evolution the former gave origin to the latter. A problem of great interest is the question of the nucleus. We know that the lowest organisms, such as Bacteria and the blue-green algæ called Cyanophyceæ, do not contain a typical nucleus. In the Protozoa the nuclei are in many cases of much more primitive structure than in higher animals. In the highly organised plants and animals the structure of the nucleus is so intricate, as is seen particularly in the process of the cleavage of nuclei, that the problem of nuclear structure cannot be longer considered a chemical one. The nucleus rather acts as a special organism in the cell. To a certain

degree plastids may be spoken of in the same way.

But the bulk of Cytoplasma shows clearly by its vital phenomena that it is principally transitory structures such as are found in other colloids, that occur there. A well-known fact is the streaming of protoplasm. Streams of liquid colloid matter wander in continual movement through the different parts of the cell, carrying with them different bodies, very frequently the chloroplasts, and in some cases even the nucleus itself. Very little is known about the reason for this remarkable phenomenon. The general impression is that surface tension plays a great part in such plasmatic streaming. By continual change of the chemical properties of the plasmatic surface phenomena may result such as are seen in streaming protoplasm. In any case, permanent structure cannot be given in freely streaming protoplasm which is continually moving in different parts of the cell. Nevertheless, numerous chemical changes of the greatest importance must take place in the streaming polioplasma under the same conditions which are found in other colloids. Just in this territory the chemistry of Life may hope to obtain results of the widest significance.

CHAPTER VI

CHEMICAL REACTIONS IN LIVING MATTER

ONE of the chief characteristics of living matter is found in the continuous range of chemical reactions which take place between living cells and their inorganic surroundings. Without cease certain substances are taken up and disappear in the endless round of chemical reactions in the cell. Other substances which have been produced by the chemical reactions in living matter pass out of the cell and reappear in inorganic nature as waste products of the life process. The whole complex of these chemical transformations is generally called *Metabolism.* Inorganic matter contrasts strikingly with living substance. However long a crystal or a piece of metal is kept in observation, there is no change of the substance, and the molecules remain the same and in the same number. For living matter the continuous change of substances is an indispensable condition of existence. To stop the supply of food material for a certain time is sufficient to cause a serious lesion of the life process or even the death of the cell. But the same happens when we hinder the passing out of the products of chemical transforma-

tion from the cell. On the other hand, we may keep a crystal of lifeless matter in a glass tube carefully shut up from all exchange of substance with the external world for as many years as we like. The existence of this crystal will continue without end and without change of any of its properties. There is no known living organism which could remain in a dry resting state for an infinitely long period of time. The longest lived are perhaps the spores of mosses which can exist in a dry state more than a hundred years. As a rule the seeds of higher plants show their vital power already weakened after ten years ; most of them do not germinate if kept more than twenty to thirty years. These experiences lead to the opinion that even dry seeds and spores of lower plants in their period of rest of vegetation continue the processes of metabolism to a certain degree. This supposition is confirmed by the fact that a very slight respiration and production of carbonic acid can be proved when the seeds contain a small percentage of water. It seems as if life were weakened in these plant organs to a quite imperceptible degree, but never, not even temporarily, really suspended.

Life is, therefore, quite inseparable from chemical reactions, and on the whole what we call life is nothing else but a complex of innumerable chemical reactions in the living substance which we call protoplasm. It must be

one of the chief tasks in explaining chemical phenomena in life to study the different chemical reactions which take place in living protoplasm.

Chemists working with lifeless material have as a rule to cause reactions by experiment, since the material does not undergo any change by itself. Comparatively few substances are readily affected by the water and oxygen contained in the surrounding air, without the help of the experimenter. The biologist, on the contrary, may watch numerous chemical reactions which take place in living matter without his aid. It is, however, difficult to study chemical reactions in life in that way, because the single results cannot be distinguished or separated from each other. Results by far more exact are obtained when in an experiment we bring together the living organism with a certain substance to see what reactions are caused. So we may watch the favourable or unfavourable influence of this substance on the living cells as well as the chemical transformation of this substance by the living organism, when we later on subject the organism to chemical analysis or when we examine the products excreted by the living cells. A great number of most valuable results were obtained by such methods. Especially the gradual change of substances taken up into living cells by different reactions may be well studied in that way.

The next step is to learn what kind of chemical

means are available in living cells to produce such results. We have now to bring together the substance which we had examined in its reactions in living cells with other substances *in vitro*. So we see whether analogous influences may be exerted by some substances contained in cells or not. We compare the artificial reaction outside the organism with the vital reactions, and are enabled to draw conclusions from our experiment for the chemical reactions in living protoplasm. Striking parallelism and resemblance are observed.

Such results, however, are incomplete, and have been obtained only with certain groups of substances. During the last decades biochemists have more and more aimed at the study of the total complex of the living cell after its death in its reactions to certain substances. The earliest experiments employed macerated tissue or whole cells of microbes under conditions which prevented decomposition by living bacteria. Salkowski twenty-five years ago allowed yeast to stand with water and some chloroform, that he might study the post-mortem transformation in this deposit of cells. It was shown that many of the contents of the living yeast cell undergo great change under such conditions, and new substances were found as products of such chemical reactions. Such chemical transformation in dead cells where microbial decomposition is excluded, is called *Autolysis*. Of late very ingenious autolytical

methods have been discovered. Instead of chloroform as an antiseptic toluol is generally used, which liquid has scarcely any injurious effect upon the substances of the cell. But, as Palladin, of St. Petersburg, has lately shown that even the grinding down does harm to many vital reactions, it is better to kill the living tissues by freezing and not to grind them. After having been frozen at 20 degrees, and having been placed in a glass with some toluol, the organs are brought back into room temperature. It is said that under such conditions more reaction takes place than when the material is ground down.

We owe to Edward Buchner, of Würzburg, another remarkable method which has the advantage of permitting us to work with liquids without any particles of living cells, as in autolytical methods must otherwise always be done. Buchner recommends the material being ground down as finely as possible, and quartz sand or silicious marl being added. The thick paste of cells and silicious powder is then pressed out in an hydraulic press under a pressure of 300 to 500 atmospheres. In this way all the cell sap is separated from the solid parts of the cells, and contains but a very small quantity of cell fragments. Even these may be removed by filtering through a Chamberland candle filter. The clear cell sap, however, still contains many substances which were hitherto known only in living intact

cells. Macfadyan and Rowland proposed a very good amendment of this method. The living organs are brought together with liquid air, and are very quickly frozen to stone-hard masses. Now they may easily be ground in the mortar. Before thawing toluol is added, and this paste of cells is ready for autolytic experiments. These methods, highly developed as they are, are continually increasing in number and value. A considerable number of reactions are now separable from general cell life, and these reactions may be studied isolated from life. Such is the aim of modern biochemistry.

Chemical reactions are bound by certain conditions. They may by some means be accelerated or diminished. The chief influences we meet with in the chemical laboratory are temperature, physical condition, separating and mixing.

Chemists are always ready to boil a test when they desire to accelerate the dissolution or reaction of a substance. It is a matter of common knowledge that chemical reactions are considerably hastened by a higher temperature. It is true that plants as a rule do not show a higher temperature than the temperature of the surrounding air. But there are remarkable exceptions. Bacteria have been found in rotting hay and other decomposing plant material and also fungi, which produce a very high degree of heat even as much as 60 degrees. Similar results were obtained with

leaves which were kept in a chest carefully isolated to prevent loss of warmth. We may consider that heat is generally produced by plants, just in the same way as by warm-blooded vertebrates. But there are no contrivances in plants to keep the temperature at a certain point above the temperature level of their surroundings. From numerous experiments we learn that plants are in their vital functions adapted to a certain average temperature. Not a few tropical plants suffer from frost and even die when the outside temperature falls below four degrees above zero. At the same temperature, on the other hand, many alpine and arctic plants have to perform all their functions in life. In tropical plants the fat of the seeds melts as a rule at a temperature of 30 to 40 degrees. It is solid at the ordinary room temperature of 15 degrees. European plants always show the melting-point of their fat not far above zero. Daily observation teaches us that plant life develops considerably more quickly in a higher temperature. Growth, respiration, and the assimilation of carbon dioxide, as well as the phenomena of movement and stimulation, reach a much higher velocity and power in a temperature of 30 to 35 degrees than in one below 20, and by far higher than in a temperature below 10 degrees.

The eminent Dutch chemist Jacobus Hendricus Van 't Hoff discovered the rule that chemical reactions are influenced by temperature with the

result that the velocity of reaction is doubled or trebled when the temperature increases by 10 degrees. This rule, well known to the chemists of our days as *Van 't Hoff's Rule or the R.G.T.-Rule*, is in practice applicable between the extremes of −50 and 300 degrees. Below and above these extremes the quotient is larger than 3 or smaller than 2. It is of great interest to see that chemical reactions in plants strictly follow the same rule. F. F. Blackman and Miss Matthæi showed that the dependence of the carbon-assimilation of leaves in sunlight upon the temperature is an exact example of Van 't Hoff's Rule. Blackman stated the same for the respiration of plants. Kanitz drew attention to many former observations of different authors which demonstrate quite sufficiently that the R.G.T.-Rule is available for protoplasma-streaming, geotropism, longitudinal growth, pulsation of vacuoles in cells, etc.

As well as the influence of temperature on chemical reactions, the influence of the physical condition of the reacting substances is an old laboratory experience: *Corpora non agunt nisi fluida.* The chemist is accustomed to dissolve the substance which is to be used in an experiment to react on other substances. The chemical course in living cells is the same. All substances destined for reactions are first dissolved. No compound is taken up into living cells before it has been dissolved. So the mineral salts of soil, the organic

compounds when being digested by the leaves of Drosera or by parasitic fungi are dissolved before they enter further chemical reactions in the living cells. Digesting is essentially identical with dissolving, or bringing into a liquid state. On the other hand, the chemist knows how to save a substance from chemical change by reactions, by transferring it from the state of solution into a solid state. This is what is called precipitation. The solid insoluble deposit of the substance now remains chemically unchanged. Metabolism in plants employs the same means. Substances which are to be stored up, such as starch, fat, or protein bodies, are deposited in insoluble solid form, ready to be dissolved and used whenever wanted for the life process. Further substances which are useless or even poisonous are easily withdrawn from the complex of chemical reactions in living protoplasm, and form a solid insoluble deposit. For instance, oxalic acid is a widespread product of oxidation in living cells which has strong poisonous properties. Oxalic acid immediately forms an insoluble compound when calcium salts are present. In reality deposits of oxalate of calcium are most common in plant cells. We may then maintain that oxalic acid is in this way withdrawn from active metabolism. Resins and essential oils in quite a similar manner are isolated and separated from the other reacting substances in living protoplasm.

To separate substances from each other by filtration or by shaking with suitable liquids is one of the daily tasks of the chemist. We must expect analogous processes to occur regularly in living cells. When filtrations are to be quickly finished, we have to use filters which have a large surface. In living protoplasm this condition is very well fulfilled by the foam-like structure, which affords an immense surface in a very small space. We have been told that fine membranes form the meshes of the network in protoplasm. These membranes have the function of filters. We know already that they are not permeable for every substance. On the contrary they dissolve and let certain substances pass through, whilst others are retained. In this way a most perfect separation is reached which may be compared with our best filtering contrivances. I may add that by adsorption the plasma membranes retain numerous substances, which process is quite analogous to precipitation and elimination from other reactions.

Finally, we have to mention the importance of procedures of mixing in chemical reactions. In ordinary laboratory practice mixing is carried out by stirring. In living cells there could not be any better contrivance for stirring or mixing than the streaming of protoplasm. There are many considerations which render it very probable that the real purpose and use of the streaming of protoplasm is the performing of this function.

CHAPTER VII

VELOCITY OF REACTIONS IN LIVING CELLS

CHEMICAL reactions are very frequently practically completed at the same moment at which they begin. It is quite impossible to measure the time which elapses from the moment when the reacting substances are brought in contact up to the moment when the end of the reaction is reached. When solutions of nitrate of silver and of sodium chloride are mixed, the two solutions immediately form the well-known white, flaky precipitate, and, provided that there is enough nitrate of silver present, all the chlorine is deposited in the form of insoluble silver salt. Most reactions used in analytic Chemistry are reactions of enormously great velocity. We comprehend, therefore, why chemists did not turn their attention to the laws of Reaction Velocity till in the last decades, when organic synthesis continually taught that there are many chemical reactions which require a considerable length of time before being ended.

Most reactions in Inorganic Chemistry take place between electrolytes—substances which are good conductors of electric currents. Many

reasons are brought forward in favour of a view which Faraday had first expressed, to explain the conducting of electric currents. The molecules of electrolytes are split, to a greater or less extent, into smaller particles which are called *Ions*. These ions carry the electricity from one pole to the other. They may be considered, as physicists believe, to be compounded of atoms and a certain quantity of electricity. The number of molecules split into ions depends upon the degree of dilution and the temperature. Strong acids and alkalis are practically entirely split up into ions when they are diluted down to 0·001 of one gramm molecule in one litre of water.

The reactions which such substances undergo may be considered as reactions between ions. We generally call them *Ionic Reactions*. We shall bear in mind that ionic reactions are carried out with infinitely great velocity. The quantity of ions contained in a solution can be calculated by determining its power of conducting electric currents. The less resistance is offered the more ions are present. The sap of living tissues always contains different ions. Therefore ionic reactions must always take place in living protoplasm.

Ionic reactions in living cells did not fail to attract much attention amongst biologists. We possess a series of excellent methods for determining the concentration of ions contained in living cells. Some of them permit us to work with

extremely small quantities of material. Especially useful are the cryoscopic methods which allow us to determine the number of ions in the volume unit from the depression of the freezing-point in comparison with that of pure water. The chief source of ions for plants is the moisture of the soil taken up by the roots. It contains, in a very diluted state, salts of sodium, potassium, lime, magnesium, iron, also hydrochloric, sulphuric and phosphoric acid. Practically only ions of these substances pass into the living plant cells. Some of these ions must disappear in reactions very quickly. Thus in living cells we cannot find potassium in the well-known reactions with platinum chloride. Traces of potassium salts immediately furnish the yellow deposit of platinum potassium chloride, but this result cannot possibly be obtained in living cells. When we burn the tissue to ashes and try the reaction again, success is certain. We may draw the conclusion that potassium salts are probably transformed in living cells into non-ionic compounds of potassium.

Very important is the formation of *Complex Ions* in metabolism. Iron salts, for example, are certainly not present in living protoplasm, but the presence of iron is always easily shown in plant ash. We can see what kind of transformation may be taking place from the reaction of copper sulphate in the presence of organic compounds.

Sulphate of copper is immediately precipitated by potassium hydroxide as a light blue gelatinous deposit of hydroxide of copper. When we add sugar solution, or solution of sodium tartrate, this deposit is dissolved into a dark blue liquid. This liquid no longer shows the characteristics of solutions which contain simple ionic copper. Therefore copper ions cannot be present. Those present are compound ions containing both copper and the organic substance.

Similar processes are, as we know, common in living cells. But living cells can even form new ions from non-ionic substances. When oxalic acid is formed from sugar or protein matter, new ions of this strong acid come into existence. Many other cases of the production of ions from non-electrolytes in living cells could be mentioned. When reactions between ions take place in protoplasm, they are not carried out in a watery liquid medium, but in a colloidal medium. It is a question, however, whether the Reaction Velocity is the same as in water. Experimental work of the last years does not leave any doubt that a colloidal medium diminishes the velocity of chemical reactions as well as the diffusion of dissolved substances. Thus it is certain that colloids of firmer consistence, such as solid gels, must retard the course of chemical reactions, even of ions. In spite of this, ionic reactions are completed in an immeasurably short time, and

practically the influence of the viscous colloidal medium in protoplasm is of very little importance for ionic reactions in living cells.

The most important substances among the carbon compounds of living matter are not electrolytes. Neither sugar, fatty bodies, carbohydrates, nor protein bodies conduct the electric current but to a very slight extent. All these substances, then, which form the greater mass of living protoplasm are non-electrolytes, and in watery solution will only form a very small quantity of ions or no ions at all. Most of the chemical reactions which take place in assimilation, digestion, and excretion are connected with such non-electrolyte organic compounds. It is, therefore, of interest to learn how great the velocity of such reactions is in comparison with ionic reactions. It is very easily shown that reactions between molecular solutions are carried out comparatively slowly, especially when the temperature does not exceed 20 degrees. So it is when starch is transformed into sugar, or protein into amino-acids, that there is no difficulty in measuring the velocity of chemical reactions. Such experimental work is very important to obtain an exact theory of the different chemical processes in living protoplasm. We define as *Reaction Velocity* the quantity of the substance transformed, measured in gramm molecules per litre, which disappears in the unit of time, viz. in one minute. If there is only one

substance transformed at the same time in the mixture of reacting substances, and if, therefore, the concentration of only this substance varies, whilst the other substances remain unchanged, the mathematical law of the process is quite simply found. The velocity of such a reaction must directly depend throughout the reaction on the acting quantity of the substance. Since this acting quantity of the substance is constantly decreasing, we see that the velocity of the reaction cannot remain the same. It must diminish in a certain ratio. Suppose 20 parts out of 100 are transformed in the first minute, then there remain in the second minute only 80 parts:

$$100-100\times0{\cdot}2=80.$$

We find for the process in the third minute the same:

$$80-80\times0{\cdot}2=64$$

In the fourth minute:

$$64-64\times0{\cdot}2=51{\cdot}2, \text{ etc.}$$

When we introduce for 100, which is the concentration at the beginning of the reaction, the general symbol C_0, and for 80, 64, 51·2, etc., subsequently C_1, C_2, C_3, . . . C_t, and for the constant factor 0·2 the symbol k, the equations are:

$$C_0-C_0k=C_1 \text{ or } C_0\ (1-k)=C_1$$

further—

$$C_0\ (1-k)-C_0k\ (1-k)=C_2$$

$$\text{or } C_0\ (1-k)^2=C_2$$

further—

$$C_0\ (1-k)^3=C_3$$

finally—

$$C_0\ (1-k)^t=C_t.$$

If, instead of 1, we take the time unit equal to $\frac{1}{n}$, we have to take k n-times smaller, and, instead of t, to write nt. The equation will now be:

$$C_0\ (1-\tfrac{k}{n})^{nt}=C_{nt.}$$

If we introduce for $\frac{k}{n}$ the value $\frac{1}{d}$, we have for $n=kd$. The equation then becomes:

$$C_0\ (1-\tfrac{1}{d})^{d\ kt}=C_{nt.}$$

The expression $(1-\frac{1}{d})^d$ can be developed according to the binomial theorem into e, the basis of natural logarithms. The equation can be formed as follows:

$$C_0\times e^{kt}=C_{nt.}$$

Or if we take logarithms:

$$ln\ C_0-ln\ C_t=kt.$$

By introducing Brigg's logarithms we have:

$$k_1=0{\cdot}4343k=\tfrac{1}{t}\ (log\ C_0-log\ C_t).$$

This expression contains values which may be determined by experiment. If we therefore find that the quotient of the difference of the logarithms in the beginning and at the end of the time of observation, measuring the time in minutes, is constant, we may be certain that only the con-

centration of one substance was changed. Such reactions are called *Reactions of the First Order*, or *Monomolecular Reactions*. Most of the reactions which take place in living cells seem to belong to this order. The determination of the substance still remaining can be made in different ways. Very often polarimetric control of the liquid in which the reaction takes place allows of a very exact conclusion on the rate at which the substance disappears. The refraction of light, or even a change in colour, can be used as a reagent of the chemical process.

In other cases the law of the reaction is a different one. We find that the reaction velocity is not directly proportional to the quantity of the reacting substance, but proportional to the square of this quantity. In all such cases, two substances are simultaneously changed in their concentration. Such a process takes place in the decomposition of esters, the compounds of organic acids and alcohols, under the influence of an alkali. There the concentration of the compound is continuously diminishing. But, on the other hand, the concentration of the alkali, which is used up in the formation of the alkali salt of the organic acid, also decreases. So it is, for instance, in the reaction between sodium hydroxide and ethyl acetate:

$$CH_3 \cdot C_2H_5OOC + NaOH = NaOOC \cdot CH_3 + C_2H_5OH.$$

Such reactions are called *Bimolecular Reactions* or *Reactions of the Second Order*. Many reactions in living cells follow the law of these reactions. Reactions of a higher order are not as yet known from living cells. We may at least be certain that the great majority of all reactions in living matter are not connected with the chemical change of more than two different substances.

In molecular reactions we generally meet with the peculiarity that the reaction is not quite completed when the reaction velocity has reached the value of O. A certain quantity of the original substance always remains and never disappears. Molecular reactions are consequently incomplete. Thus a small quantity of cane sugar remains unchanged when cane sugar is split by means of diluted hydrochloric acid, and in the same way some quantity of the unsplit ester remains when we split it by means of acid into alcohol and acid. This remarkable phenomenon becomes quite clear if we suppose that the two reactions always take place in opposite directions. Simultaneously with splitting up begins the synthetical reaction, and synthesis increases in proportion as the splitting of the compound advances. The velocity of the splitting process decreases at the same rate as the velocity of the recomposing process increases. At a certain time both processes have the same velocity. No further change takes place in the chemical system, provided that nothing is taken

away nor added. The characteristic stage of equilibrium of the reaction has been reached. We express this rule by writing the chemical equation connected by a double arrow instead of the sign of equation :

$$C_2H_5OH + CH_3COOH \rightleftarrows C_2H_5OOC\cdot CH_3 + H_2O$$

Ethyl alcohol + Acetic acid $\rightleftarrows$ Ethyl acetate + Water

or $C_6H_{12}O_6 + C_6H_{12}O_6 \rightleftarrows C_{12}H_{22}O_{11} + H_2O$

Glucose + Fructose $\rightleftarrows$ Saccharose + Water

This theory involves the condition that all these reactions may be reversed under certain circumstances. It only depends upon the external conditions in which direction the situation of the stage of equilibrium is displaced, either in the direction of composition or in the direction of decomposition. We may draw the further conclusion that many chemical processes in living cells may obey this kind of law. Under certain circumstances cells may contain more grape sugar and fructose, under other circumstances more cane sugar. Only chemical or physical agents influence this relation, and we need no longer take refuge in mysterious " vital forces " when we want to explain these facts. Just such chemical reaction-complexes occur most frequently in living cells. The digestion and dissolution of organic matter in the cell on the one hand, and the storage of organic matter on the other, must be ruled by analogous

laws. When there is a scarcity of food, the digestion of starch or protein must yet be continued until the concentration of the disintegration-products has reached a decisive point. But has the concentration risen above a certain point, the process of recomposition becomes predominant, with the result that storage of starch or protein takes place.

Such regularity can only exist as long as no reaction products are taken away or added. When we remove the products of dissimilation, e.g. the sugar produced in the decomposition of starch, the splitting process continues and does not cease until the whole stock of starch has disappeared and has been transformed into sugar. Working upon this principle we can deprive seeds entirely of starch, even the isolated endosperm when the embryo has been removed. The seeds are fastened each upon a small cylinder made of plaster of Paris, which is placed in a dish filled with water. The principle of such an experiment is quite the same as that which is followed in the emptying of leaves of starch during the night. In the process of respiration and growth at night the growing plant consumes considerable quantities of sugar. At the end of a warm summer day leaves are full of starch, and allow a constant stream of sugar solution to be directed to the places where sugar is consumed. By this process the decomposition of starch grains is continually assisted, since all

the sugar which has been formed from starch is immediately removed.

The contrary effect, viz. that further formation of compounds is hindered when the storage of this compound has reached a certain stage, is also a frequent phenomenon in living organisms. When leaves are cut off from the branch and are exposed to sunlight under favourable conditions of life, for a certain time they continue their assimilation of carbon dioxide, and starch is formed to a considerable extent. Even more starch is stored in such leaves than in normal leaves which have not been separated from the plant. But, after a time, carbon dioxide assimilation diminishes and ceases entirely. The concentration of sugar in the leaf cells becomes too great and the assimilation process is hindered by the reaction products.

The mechanism accelerating and ceasing reactions in living cells is very often simply regulated by the general laws of reaction velocity, and we need not assume any special power of living protoplasm. The next chapter will touch on one of the most important influences on the reaction velocity, and will show that living cells possess most effective means to accelerate reactions and to cause surprising chemical results.

CHAPTER VIII

CATALYSIS AND THE ENZYMES

IN the beginning of the last century chemists made the acquaintance of a series of remarkable phenomena, which were caused by finely divided metals, particularly by platinum in the form of the so-called *Platinum black.* A mixture of oxygen and hydrogen immediately explodes when it is brought in contact with platinum black. Common coal gas inflames when brought in contact with finely divided platinum. Sulphur dioxide is by the same agency quickly oxidised to sulphuric acid. Hydroperoxide is rapidly split into oxygen and water when in contact with platinum black. In all these cases the quantity of platinum black is not diminished after the reaction, and the products of the reactions are never any of the platinum compounds. Similar effects were later on known from sulphuric acid in its influence on the formation of ethyl ether or sulphuric ether from the common ethyl alcohol. Here, too, no sulphuric compound is formed. Ether is often called Sulphuric Ether for the reason that it is prepared by means of sulphuric

acid. Its formula $\begin{matrix} C\ H_5 \\ C_2\ H_5 \end{matrix} > O$ does not contain any sulphur. It is formed from alcohol simply by loss of water: $2\ (C_2H_5OH) - H_2O = (C_2H_5)\ O$. No sulphuric acid is consumed in this process. Such remarkable reactions have become known in continually increasing number. Since the effect of the metal or the sulphuric acid seems to be caused merely by contact, the German chemist Mitscherlich proposed to call such effects *Contact Effects*. Mitscherlich recognised a very important fact in many of such contact reactions, viz. that in these the large surface of finely divided contact substances must play an important part. The famous Swedish chemist Berzelius, who took a great interest in these phenomena, believed that a peculiar force is exerted by contact substances. He called that force *Catalytic Power*. The name *Catalysis* has since been generally accepted. Catalytic reactions soon became most important for biology. Just a century ago Kirchhoff, of St. Petersburg, found that starch is transformed into grape sugar by the working of mineral acids. It was known to him that no acid is consumed in this process. In 1833 Payen and Persoz in Paris made the discovery, which has had far-reaching consequences, that germinating seeds contain a peculiar contact substance, which transforms starch into sugar. This substance they named *Diastase*. In quick succession similar reaction

effects were recognised in the formation of prussic acid from the so-called amygdaline in germinating bitter almonds, in the formation of the sharp essential oil in germinating mustard seed, and, finally, in protein digestion in the stomach of man and the higher animals. Berzelius did not hesitate to express his opinion that catalytic reactions will probably one day represent the most important part of the chemistry of living cells.

At present, indeed, we have at our disposal a surprisingly great mass of facts which illustrate the general occurrence of catalytic substances in living cells and the overwhelming importance of catalytic reactions for chemical phenomena in life. I shall try to explain the position of our knowledge in the following pages as well as it is possible to do in a narrow space.

To Ostwald, of Leipzig, we owe a very ingenious and practical definition of catalytic reactions and catalytic power. Substances which act as *Catalysers*, as we now call them, usually exert their influence upon a suitable substance, even when applied in very small quantities. As a rule one part of the effective substance may transform many thousands, even millions of parts of the substance undergoing the catalytic change. But during the reaction the quantity of the catalyser does not diminish. For instance, when splitting up cane sugar into glucose and fructose by means of acid, the acidity of the solution does not show

the slightest alteration. Finally, as we have already seen, no trace of the catalyser appears in the final products of the reaction. Reactions which show these characteristics we call *Catalytic Reactions.* The enormous power of the slightest trace of a catalytic substance strongly reminds the biologist of the effects of stimulation in animals and plants. Even here a slight stimulus very often produces a surprisingly great effect. Physiologists know that there is as a rule no mathematical relation between the energy of the stimulus and the energy which becomes manifest in the reaction. For such physiological phenomena the expression *Release Action* was used. Pfeffer tried to compare such processes with the mechanism of a machine which may be set working by touching an electric button or a spring. Indeed, in both cases the releasing action is not at all comparable with the resulting action. May catalytic effects also be called release actions? Physiologists sometimes did so, but there is no doubt that there are reasons enough for drawing an exact distinction between the two results. When the trigger of a gun is touched, it does not matter whether more or less power is applied. The energy produced by the explosion is always the same. In catalytic reactions, on the other hand, the quantity of the catalyser employed is of great importance as regards the amount of the reaction effect. Between certain limits one may even consider the reaction

effect as proportional to the quantity of the catalysing substance. So the acceleration of the splitting of cane sugar by acids was found to be directly proportional to the concentration of the acid applied. Another difference is shown by the experience that release effects in processes of stimulation in plants or in animals do not occur without a stimulus. But catalytic reactions, as it seems, are not strictly dependent for their existence on the presence of the catalyser. For a series of reactions it has already been stated that the reaction takes place even without the catalyser being present, yet, it must be admitted, slowly.

We come to the conclusion that the catalysing substance is only an accelerating agent, but not an agent without which the effect does not take place at all. This is very important for an exact understanding of catalysis effects. If we find it desirable to compare the catalyser with any mechanism in an engine, we cannot compare it with a releasing contrivance, but we may rather find a resemblance between the effect of train-oil on the smooth going of the engine and the accelerating effect of a catalysing substance.

Hitherto only accelerating catalysis has been spoken of. Some effects on chemical reactions have been found which seem to have the contrary of an accelerating catalytic influence. The oxidation of sulphurous acid, for example, can be

very much retarded by traces of glycerin, mannitol, or other organic compounds. The luminosity of phosphorus is diminished or hindered by the presence of turpentine, ether, or alcohol. Probably all such influences are based in the working of these agencies on a catalysing substance. In the first case which we have mentioned, traces of copper contained in the common distilled water of our laboratories exert a catalysing influence upon the oxidation of the sulphite of sodium. Organic substances, for example mannitol and glycerin, are inclined to form compounds of copper and so they remove the effective catalytic agent from the water, and diminish the velocity of the oxidation of the sulphite of sodium.

We owe to Bredig, of Zurich, the exact knowledge of the retarding influence of traces of prussic acid, sulphide of hydrogen and some other substances on the catalytic reaction of platinum black and hydrogen peroxide. There is no doubt that prussic acid or hydrogen sulphide change the surface of the platinum, for they cover it with a layer of platinum cyanide or sulphide. So the platinum surface which exercises the catalytic power is very considerably diminished. By decomposition of the cyanide layer the pure platinum surface can be restored and the catalyser becomes active again. There is an interesting parallelism between these phenomena and the poisoning of living cells by cyanide or sulphide,

which made Bredig call such retarding substances *Poisons for catalytic and enzyme effects.*

A very interesting result in chemical reactions is often given by the phenomenon that the catalysing substance is formed by the reaction itself. Pure copper metal is very much less soluble in quite pure nitric acid than in nitric acid which contains a little nitrous acid. The latter acid has a catalytic influence on the process of the dissolving of copper. Now some small quantity of nitrous acid is always formed by the reduction of the nitric acid during the process of dissolving copper. We therefore see that, after a certain time, the copper dissolves much more quickly than in the beginning. Such a catalysis is called *Autocatalysis.* We may compare it to the influence of heat on the dissolution of sodium hydroxide, during which process heat can be produced by the process itself.

Catalytic substances sometimes, in the same way as platinum black or acids, may influence a large number of reactions. Acids particularly are quite usual catalytic substances which affect nearly every kind of reaction.

It is a very important fact that the final equilibrium in the reaction is as little altered by the presence of the catalysing substance as that the order of the reaction is changed. Consequently the catalytic influence does not extend but to the reaction velocity. Catalytic reactions are of the greatest importance for chemical phenomena in

living matter. We may even say that all the main reactions in the different processes of digestion, in respiration, in the metabolism of carbohydrates, fats and proteids are ruled by catalytic influences. No chapter of biochemistry during the last period of development in biology has become of greater significance than the theory of catalysis in living protoplasm, or the knowledge of the *Enzymes*.

The word *Enzyme* has not been used until recently. Formerly the expression *Ferment* was generally applied to signify the cause of the remarkable chemical changes which are so highly characteristic of life. *Ferment* or *Fermentation* was directly derived from alcoholic fermentation. The word was intended to signify the generation of gas, of foam bubbles filled with gas, and it should remind us of the resemblance to boiling liquids: *ferveo*, boil, bubble. Figuratively, *fermentation* was applied to chemical changes in organic bodies under organic influences. There was no marked distinction made between fermentation and rotting or decomposition. Generally fermentation and putrefaction were spoken of as being the same. The first great discovery in the territory of fermentation was made by Theodore Schwann in Belgium and Cagniard Latour in France. It was shown that the deposit consisting of yeast in fermenting sugar solution was of vegetable nature, not a product of fermentation as was formerly often

believed, and that it was the active cause of the fermenting. From that time yeast has been placed in the plant system among the fungi. A little later Kutzing was able to show that the cause of acetic fermentation was also a microscopic plant, belonging to the bacteria. It is still well remembered what great services Louis Pasteur rendered to the knowledge of microbes which cause different fermentations. In consequence of these discoveries the name of ferments was transferred to the microbes causing fermentation. I have already taken the opportunity of mentioning a further wonderful discovery of the remarkable third decade of the last century. I mean the isolation from germinating seeds of a substance which is able to transform starch into sugar. Payen and Persoz first showed that extract of malt contained a certain substance, soluble in water, and which was precipitated by alcohol, which causes the starch grains to dissolve and induces the formation of sugar from starch. The two French scientists even showed that this substance, to which was given the name of *Diastase*, immediately loses its power when boiled. Theodore Schwann, at about the same time, discovered that from the mucous membrane of the stomach there can be extracted a substance which is soluble in water or glycerine, and which acts very effectively upon albuminous compounds, quite in the same way as in digestion the living organ changes

albumin. This substance was called *Pepsin.* In rapid sequence followed the discovery of *Emulsin,* which splits up the amygdalin contained in almonds to prussic acid, benzaldehyde and grape sugar ; the discovery of *Myrosin* in mustard seeds, which produces mustard oil ; later on the discovery of *Invertin* in yeast, which cane sugar splits into its sugar components ; *Trypsin* in the pancreas gland of quadrupeds, which rapidly splits up albumin to amino-acids. Many other discoveries were made later on, in connection with which I only mention the important statement of Schoenbein in Basel, that oxidising effects are caused by substances which are soluble in water, precipitated by alcohol and destroyed by boiling. All these substances exercise their activity, even when applied in very small quantities. They are all of organic origin, never found in inorganic nature, and not to be gained by chemical synthesis. We do not wonder that such effects caused by diastase and the other substances mentioned were not sharply distinguished from the microbial processes of fermentation or decomposition. We indeed see the expression *Fermentation* used for both kinds of phenomena. It was found sufficient to speak of *Soluble Ferments* and of *Microbic Ferments.*

Kuhne, of Heidelberg, was the first to propose to change the nomenclature and to avoid speaking of ferments. He clearly recognised that even the

microbes cannot act otherwise but by production of substances which must be regarded as Soluble Ferments. Consequently the name of *Enzymes* was introduced for soluble ferments. We know that all enzymatic processes depend upon the production of such substances. All the processes which were formerly believed to be exclusively connected with living protoplasm are due to substances of the group of *Enzymes*.

In this direction, particularly the discovery of Edward Buchner, of Würzburg, then in Munich, was of the greatest importance. It was shown in 1894 that the power of fermenting sugar in yeast is by no means inseparably connected with cell-life. When yeast is carefully ground down, so that every cell is sure to be cut through or squeezed, and afterwards the paste is pressed by means of a powerful hydraulic press, a yellowish liquid is obtained which still possesses the full property of forming alcohol and carbon dioxide from grape sugar. Buchner succeeded by filtration in freeing this liquid from every trace of living cells or their fragments, so that there could not be any doubt that no living protoplasm was present. Further, he demonstrated that the alcohol-forming agent was soluble in water, precipitable by alcohol, and very easily destroyed by heat. So alcoholic fermentation was separated from yeast-life and the perspective was opened, that many other processes of decomposition or disintegration of

substances in living protoplasm may be caused by enzymes, but not directly by the living matter itself.

But it is true that in some cases substances which are responsible for enzymatic actions cannot be extracted from protoplasm. The expressed sap proves ineffective and no means are known for separating the hypothetical enzyme from the protoplasm. In such cases it is, however, possible to kill the protoplasm without destroying the enzymes. Here, too, Buchner was the first to show useful methods. He succeeded in killing cells by means of acetone or ether without damaging the enzyme. So killed yeast-cells were obtained which possessed in a high degree the power of acting on sugar. In the same way Buchner prepared the bacteria of milk fermentation and of acetic fermentation. The cell-bodies were completely dead, but nevertheless it was possible to cause fermentations by specimens of these bacteria. We may consider that such experiments fairly prove the existence of specific enzymes which are responsible for the fermentation effects by the living cells. It is difficult to explain the reason why the enzymes in these cases cannot be separated from protoplasm. They may be entirely insoluble, or may at least diffuse through membranes only with difficulty, or adsorption effects may play a part in such cases.

Investigations of later years have shown dis-

tinctly that every cell contains such enzymes which are not to be extracted from protoplasm, and which never diffuse from intact living cells. Such enzymes were named *Intracellular Enzymes* or *Endo-Enzymes.* Other enzymes, such as the cane sugar inverting enzyme of yeast, or the digestive enzyme of the mucous membrane of the stomach are abundantly secreted and consequently may be obtained without difficulty in any quantity from living tissue. These are the enzymes which we call *Secretion Enzymes.*

We understand that chemists were very anxious to isolate pure enzymes and to study the properties of these most remarkable substances in the hope of being able to explain why they act in that way. In spite of the very advanced technical achievements of experimental chemistry, it was not possible to prepare a pure enzyme, not even in one case. The difficulties of preparation are very great. All enzymes have proved to be typically colloidal substances, and they readily show alterations of their properties, coagulate, are destroyed by heat, show a high degree of adsorption of other substances, and are mixed with very many similar colloidal substances, so that the chemist, in his endeavour to separate the effective agent from its companions, loses more of it the longer he treats it with reagents, and often finally has before him a white powder, looking quite satisfactorily pure, but of much

less activity than the original enzyme. We must confess that it is at present impossible to say whether all enzymes belong to the class of albuminous substances, as in many cases seems probable, or whether enzymes may be of different chemical structure. It is not even certain whether all enzymes contain nitrogen.

As far as we know all enzymes are distinctly colloidal substances. No enzyme survives boiling even for a short time. Although there is great uncertainty about the chemical nature and relation of enzymes we possess much knowledge of the action of enzymes, which is doubtless the most interesting part of their characteristics. At the first glance we must feel reminded of catalytic reactions. Berzelius made no difference between enzymes and catalytic substances. As well as being catalysers the enzymes show strong actions even when applied in but very small quantities. It was stated with regard to a series of enzyme reactions that the quantity of the enzyme is not diminished in a perceptible degree during the reaction. We know further that the enzyme never appears among the products of a reaction, quite as in catalytic reactions. Finally, it is most probable that the reactions which are caused by enzymes do not entirely depend for their existence upon the presence of the enzyme. They are continued and take place, though very slowly, even when the enzyme is not present. We see that

the chief characteristics of catalytic substances and of enzymes agree exactly. We must in consequence of this consider enzymes to be catalytic agents.

But there are a few very remarkable and sharp differences between the two groups of substances. Most of the catalysers we have spoken about extend their sphere of action over a large number of substances. Acids, for example, are able to catalyse all kinds of reactions. Quite a different behaviour is met with in enzymes. As a rule enzymes are effective only in one reaction. Invertin does not act upon anything else but on cane sugar, emulsin only upon amygdalin. Their sphere is, as we see, very limited. Another peculiarity of enzymes is their colloidal nature and their inability to resist boiling temperature. There is little doubt that both properties are connected, and that the sensibility to heat is due to coagulation of colloidal solutions. We may therefore say that enzymes are catalytic substances of a limited field of action, of colloidal nature, and very little resistent to heat. We must still add that enzymes are formed only in living matter. Finally, one important property of enzymes is this, that in the blood of animals which have had some enzyme solution injected into a vein, peculiar substances are formed. These have the power of hindering the enzyme action when a little of the blood serum is added to a mixture of the original enzyme solution and the substance on which the enzyme

is otherwise effective. We call these remrakable substances *Anti-Enzymes*. Only real enzymes cause the formation of anti-enzymes in animal blood, and this reaction is highly characteristic of true enzymes. It is important to know that each anti-enzyme acts quite specifically only upon that enzyme which was injected into the vein, and upon no other.

Enzymes are as a rule easily soluble in water, in salt solutions, or in glycerine, but yet some are known which are scarcely soluble in water, such as the fat-splitting enzymes and that which acts upon malt sugar. They pass slowly through animal membranes. Adsorption phenomena are very marked in enzymes. All are greedily taken up by coal or by flakes of blood-fibrin. We prepare enzymes roughly from watery solutions by precipitating with alcohol. Sometimes they may be extracted with glycerine. In a somewhat purer state they are obtained by precipitation with a strong salt-solution, particularly when repeatedly precipitated. When they cannot be dissolved in water, the cells are ground down carefully and some toluol is added to the paste. Such toluol preparations show most of the reactions of the endo-enzymes. It is true that toluol autolysis is not free from disadvantages.

As a rule the cell-paste is effective on a great number of substances. A paste prepared from root-tips is able to split up starch and cane sugar,

as well as albuminous bodies ; it acts on oxidisable substances and splits up fats. The same was found of paste formed from animal liver. Most probably a large number of different enzymes occur in the narrow space of each cell. It is astonishing to see how all these actions can be exerted at the same time without disturbing each other and how exactly regulated they are. We have here another argument for the subtle structure which protoplasm must possess, that every substance of the cell is kept in its proper place, and cannot mix with the others. It is an important fact that enzymes of a certain kind are not formed by the organism under all conditions. That was shown distinctly in experiments on moulds. The common mould, *Penicillium glaucum*, when cultivated on starchy material produces in abundance an enzyme which acts on starch, the so-called *Amylase* or *Diastase*. But if the fungus is kept on starch-free food, it has been found that it does not contain any diastatic enzyme. The latter is only immediately and abundantly produced when starch is added to the culture medium. *Penicillium* even produces an enzyme which acts on wood substance, as I once showed. But such an enzyme is only produced if the fungus is cultivated on wood and not upon any other substance. We must conclude that the formation of enzymes in the organism underlies some regulations, and that it is a purposive process in life.

Now comes the question what enzymes may be formed of. Very little has hitherto been discovered about the origin of enzymes. Only a few hints are given by a series of experimental results. In a number of cases it has been stated that extracts from cells do not contain ready and effective enzymes. But when they are treated with very diluted acetic acid or other milder chemical agents they begin to show distinct working on fat or albuminous matter or on starch. Therefore the supposition was arrived at that the fresh cell-extract contained the natural mother-substance of these enzymes, and that this mother-substance was able to furnish the enzyme itself by artificial transformation. The original substances were called *Pro-Enzymes* or *Zymogens*.

Studies on the pancreatic ferment in animal intestines have shown that the fresh pancreatic juice does not act on protein bodies. But when it is brought together with the intestinal liquid it begins to act most energetically on proteins. The intestinal liquid entirely loses its activating effect when boiled. The activating substance must consequently be destroyed by heat quite as enzymes are. Other experiments showed that the activating substance of the intestinal sap much resembles a true enzyme, and it may be called an *Enzyme activating Enzyme*, or *Kinase*.

Enzyme effects are assisted also by many other substances. We know the great influence which

is exercised on the protein-splitting enzyme of the stomach-secretion by hydrochloric acid or another acid in sufficient concentration. Many of the enzymes of plant cells are favourably influenced in their action by acids quite in the same way. The pancreatic enzyme on the other hand shows a contrary behaviour. It is supported by diluted alkaline solutions. Very remarkable is the activating effect on the fat-splitting enzyme of the pancreatic gland exerted by the organic acids of bile, the glycocholic and taurocholic acid.

Such activating effects are extremely widely spread in the part which enzymes play in the life-process. One sees how these enzyme effects may be regulated, strengthened, and weakened, as the effects are required.

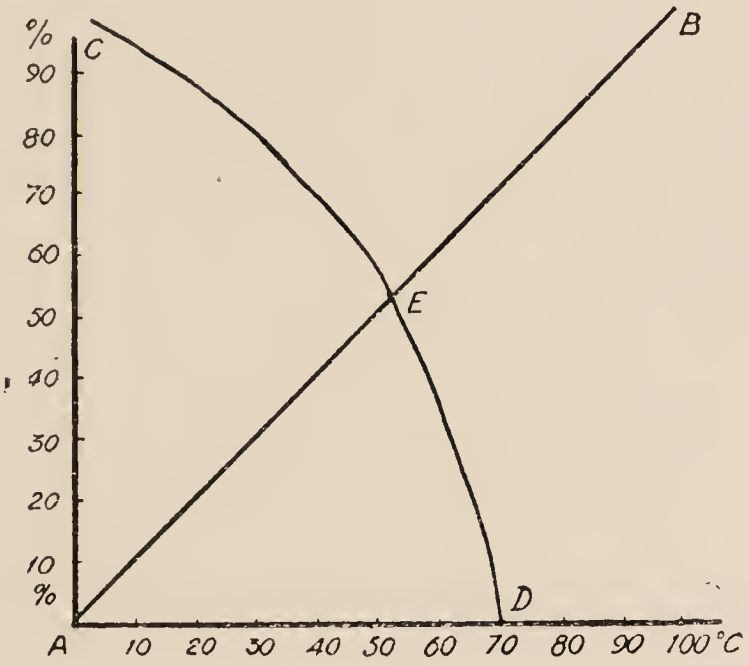
Many chemical substances hinder enzyme reactions in a most characteristic manner. Stronger acids or stronger alkalis generally diminish the enzyme effects as also alcohol, formaldehyde, cyanide of potassium, aromatic substances, and many inorganic substances, such as the salts of heavy metals, iodine, sulphurous acid, etc. Such a paralysing influence is not only exercised by these substances, but the living cell is able to produce special substances, which are destroyed by heat, which are effective in very small quantities, and which paralyse enzyme reactions. We have spoken of these already as the *Anti-Enzymes*. Anti-enzymes are doubtless produced in the

normal metabolism of plants and animals. I found a very interesting case of an anti-enzyme in root-tips after geotropic stimulation. This anti-enzyme acts on oxidising enzymes, and is able to reduce their effect considerably. Quite distinct is the specific nature of anti-enzymes. The anti-enzyme of geotropically stimulated roots of maize does not alter the anti-enzyme effects of oxidising enzymes from the bean or sunflower. On the other hand, the anti-enzyme of the bean-root acts on the enzyme of other leguminous plants only. The specific nature of anti-enzymes is met with in a similar way in the animal anti-enzymes which are produced in the blood when enzymes are injected into the venous system. As we have already mentioned, anti-enzymes are formed under such conditions, which paralyse only the enzyme which was injected, and no other.

Just as a high temperature has a great influence upon the velocity of reactions catalysed by substances of inanimate nature, the enzyme reactions are likewise considerably accelerated, when the temperature is raised. Van 't Hoff's Rule seems to be followed even in enzymes. The reaction velocity is doubled or trebled when the temperature is raised by 10 degrees. But it is true that this rule is only found for certain intervals of temperature. Besides its accelerating effect on the velocity of the enzyme reaction, a higher temperature strongly influences the velocity of the

disintegration of the enzyme. The higher the temperature the more unstable are enzymes. At a temperature of over 60 degrees enzymes are rapidly decomposed, many become immediately inactive when they are heated up to 63 to 65 degrees Celsius. We therefore understand that there probably exists a certain temperature at which the enzyme work is best done, viz. one at which the accelerating effect of the temperature is strong enough to finish the reaction very quickly, and where the enzyme destroying effect of the temperature is not so strong as to paralyse the temperature effect on the velocity of the reaction. This relation can be shown graphically by two curves. The line AB shows the acceleration of the enzyme reaction by the rising temperature. We take it for granted that this influence is directly proportional to the temperature. The curve CD shows the destruction of the enzyme by the temperature rising. This influence as far as we know is not simply proportional to the temperature. Suppose the quantity of the enzyme at 0 is 100, and the quantity at 70 degrees is 0, we have to draw the curve CD. So we recognise that the optimum of the effect lies between 50 and 60 degrees. Only about 55 per cent is active, but the strong acceleration of the reaction velocity neutralises this diminution. At 60 degrees about 40 per cent of the enzyme is active. Consequently, this minus is to be subtracted from the ordinate,

and the resulting curve of the enzyme effect slightly approaches the axis of abscissas. At a higher temperature the quantity of the active enzyme decreases rapidly, and so does the resulting effect, which becomes 0 at 70 degrees.



Such superposition of two curves causes the culmination of the resulting curve in E. In practice it is not advisable to use too high a temperature for enzyme reactions. A medium temperature is in most cases the best. We shall not be surprised to see that this so-called *Optimum* of enzyme reactions coincides with the temperature which is most favourable for the life process. F. Frost Blackman, in a series of most interesting papers, showed that the dependence of different life

processes on the temperature obeys a similar rule to that of enzyme reactions. Whenever we find an *Optimum* of a certain vital function at a certain temperature we must think of the crossing of two kinds of influences. One of these influences is the accelerating effect of the temperature on chemical reactions, the other the destructive effect of higher temperature on the active substances of living cells. We only have to add that most of these active substances belong to the enzymes.

It is important that the equilibrium of Enzyme reactions is not altered by temperature. Van 't Hoff has explained this fact. Enzyme reactions cause neither a great production nor a great consumption of heat. All reactions of such character, of a comparatively small caloric change are not affected in their equilibrium by temperature. Therefore the constant of equilibrium in enzyme reactions is not dependent on temperature.

Bright sunlight is very harmful for enzymes, and rays of light destroy them very quickly. Especially the ultraviolet rays act particularly injuriously on all enzymes hitherto examined.

Very interesting relations exist between the concentration of the enzyme solution and the enzyme effect. We have related that many catalytic reactions follow the law of monomolecular reactions. So, for example, the destruction of hydrogen peroxide by platinum sol, or the splitting up of cane

sugar by diluted sulphuric acid, are reactions of the first order. In every moment of the reaction its velocity is directly proportional to the quantity of the substance yet unchanged, and directly proportional to the concentration of the acid.

Quite similar ratios were found in enzyme chemistry. The cane-sugar-splitting enzyme of yeast, called Invertase, and Amylase or the starch-dissolving agent in seeds, act in the same way. Between certain extreme limits the effect is directly proportional to the concentration of the enzyme. So it is possible to calculate the quantity of invertase or of amylase in a solution, when a standard solution of the same enzyme is used. Pepsin of the stomach showed a different result. In Prague, in 1885, Schutz discovered that the amount of protein digested in a certain time is not proportional to the quantity of the enzyme itself, but proportional to the square root of the quantity of the enzyme. This rule has often been confirmed. But it was only a couple of years ago that Arrhenius, of Stockholm, explained this remarkable law. If we consider that the determination of the enzyme effects is made in the first stage of the enzyme action, we may assume that the quantity of the transformed albumin is very small in comparison with the quantity of the albumin not yet decomposed. We can therefore suppose that at the beginning of the reaction the quantity yet unaltered is constant.

If k is the constant of the reaction velocity, x the transformed albumin, M the not yet decomposed albumin, then the equation can be written as $k = x \cdot M$, or $M = k \cdot \frac{1}{x} \cdot (1)$.

According to the rule of Schutz, the ratio of the transformed albumin x to the time wanted for the transformation is—

$$x = k_1 \sqrt{t} \quad \text{or} \quad x^2 = k_1^2 \cdot t.$$

If we differentiate, we find

$$2x \cdot dx = k_1^2 \cdot dt \quad \text{or} \quad \frac{dx}{dt} = \frac{k_1^2}{2} \cdot \frac{1}{x}.$$

As long as M is proportional to the reaction velocity (1) Schutz' rule must therefore be valid.

Another question is whether even enzyme reactions are of the first order, that is, are monomolecular reactions or not. We see that the question is of great importance. In the case of the enzyme reaction being really of the first order, we know that only one substance in its concentration is altered during the reaction. And that cannot be any other than the substance on which the enzyme is acting. Consequently the enzyme concentration itself remains constant. In this way we obtain the proof for the identity of enzyme reactions and catalytic reactions. As early as 1890 excellent papers were published by O'Sullivan and Thompson on the reaction between cane sugar and invertase. These authors came to the conclu-

sion that the reaction follows the law of monomolecular reactions. This theory was by no means generally accepted. French and German scientists of great weight denied that the law of the reaction is simply the law of mass effect, and empiric formulas were calculated which sufficiently agreed with the course of reaction observed. It is to Hudson that we owe the proof that O'Sullivan and his collaborator were quite right. The able American chemist found that the chief mistakes in such investigations are caused by the circumstance that grape sugar continually changes its action on polarised light when just split off from cane sugar. This property of glucose is called mutarotation. Hudson very cleverlyavoided this source of error by adding some alkali to the solution before the polarimetric determination was made. Thus the state of equilibrium is at once reached in the rotation and the determinations of glucose can be made without any difficulty and with full certainty. In this way it was clearly shown that the inversion of cane-sugar by invertase is just as much a monomolecular reaction as the parallel reaction of cane-sugar inversion by means of acid. Investigations were made on fat-splitting enzymes which showed the same law, but the results of others were different. But another enzyme very clearly follows the law mentioned above. That is the catalase, which splits up hydrogen into water and oxygen. Finally the

tyrosin oxidising enzyme of plant cells was found to follow the law of monomolecular reactions. Even if we do not yet possess clear knowledge about other important enzyme reactions, these results are most remarkable. Hope is given us that some more enzyme reactions are quite identical in their mechanism with catalytic monomolecular reactions. Since we have seen that Schutz' Rule can be simply explained, and is by no means peculiar to enzyme reactions, we believe that it is very probable that enzymes are nothing else but organic catalytic substances without any peculiar property. Complications, it is true, are frequently produced by the colloidal properties of enzymes, which cause the great instability of the enzymes. In most cases the quantity of the enzyme is diminished at the end of the reaction because of the destruction of a certain amount of enzyme in other reactions which occur besides the main reaction. It is easily understood that this must lead to important differences from the law of monomolecular reactions.

Finally we have to touch on the question of the specific character of the different enzymes. A priori we do not know whether one and the same enzyme cannot catalyse different reactions. But many reasons can be given for the supposition that by far the greater number of the enzymes act upon only one substance. Although most

living cells show different enzyme effects, we find a certain variety in their combinations, and never find two or more enzyme effects inseparably connected in any case. So in germinating seeds we very often observe catalytic effects both on cane sugar and on malt sugar. In other cases these two effects are strictly limited to two different cell-species. In yeast *Saccharomyces cerevisiæ* acts very effectively on cane sugar, but not on malt sugar, whilst *Saccharomyces Marxianus* only acts on malt sugar. When we prepare a watery extract from both species of yeast we can easily convince ourselves that even there only one of the two enzyme effects is exerted. By *Marxianus* only the splitting of maltose, by *Cerevisiæ* of saccharose. We cannot doubt that these two enzymes are different substances. Many more difficulties arise when the enzymes cannot be separated from the cells and the enzyme effects are watched only in the paste of ground-down cells. There it is often impossible to say to what number of enzymes all these effects should be attributed. All in all one feels at present inclined to indulge in the opinion that each single effect corresponds to one certain enzyme. We are justified in doing so, since many enzyme preparations have in the course of time proved to be mixtures of different enzymes. It was well known that preparations of starch-attacking enzymes gave in most cases a blue reaction with guaiacum resin.

Then amylases have been met with which did not show this guaiacum reaction. Finally extracts were obtained from plants which only gave the guaiacum reaction but did not act upon starch. So the conviction was arrived at that the blue reaction with guaiacum and the starch-decomposing effect belong to different enzymes, which, it is true, very often occur together.

Since we know very little about enzymes, except of their action, it is natural to found the system of the enzymes upon the kind of reaction which each carries out. Thus the nomenclature of enzymes nowadays is generally taken from the enzyme action. It was found convenient to compose the name of the enzyme with the ending *-ase*, taken from the first described and isolated enzyme, the *Diastase*. As the root of the name of an enzyme, is taken the name of the substance which is decomposed by this enzyme. So we shall call starch-decomposing enzymes, from *amylum*, starch, *Amylase;* similarly the enzyme acting on cane sugar *Saccharase*, etc.

The chemical characteristic of the enzyme reaction or the special decomposition caused by the enzyme is very different. In many cases the action consists, as in cane-sugar inversion or starch dissolution, merely in an addition of water, which is followed by a splitting up of the substance. Chemists generally call such effects *Hydrolysis*. All enzymes which provoke hydrolysis may be

united in the chemical group of *Hydrolytic Enzymes* or *Hydrolases*. Among these enzymes different sub-orders may be distinguished according to the chemical order to which the substance attacked belongs. If esters or compound ethers of alcohols and acids are decomposed by enzymes the latter may be called *Esterases;* if they act on carbohydrates, *Carbohydrases;* if they act on fats, *Lipases*, etc.

Other enzymes have the peculiarity that they split off the group NH_2 from nitrogen containing organic substance. Since this group is called the Amido-group, the enzymes must be named *Amidases*. To such enzymes belong even the most important enzymes which act on proteids, the *Proteases*. Certain enzymes produce precipitations in albuminous solutions by hydrolysis. We call them *Coagulases*.

Another group is characterised by the oxidising effects of its enzymes. These are the *Oxidases*. Their counterpart is formed by the *Reductases*, or reducing enzymes. Further are known enzymes, which split off carbonic acid from organic acids. We call them *Carboxylases*. Perhaps even the enzyme which causes the alcoholic fermentation by yeast, the *Zymase*, belongs to these.

For physiologists it is rather more interesting to distribute the enzymes according to their physiological significance in the living cell. Following the physiological principle, we may distinguish

three large groups of enzymes: enzymes in the service of the assimilation of food and of digestion, enzymes employed in respiration, and those employed in dissimilating processes partly forming the so-called end-products of metabolism. We may maintain that all decomposing processes connected with the assimilation of food are ruled by enzyme reactions. The end of all these reactions is to form from the substances occurring in food the primitive stem-substance, such as glucose from the carbohydrates or amino-acids from albuminous substances. Each cell contains such enzymes, and is able to reconstruct its substances from the fundamental organic groups which are formed from the food by a host of enzyme reactions. In consequence of this, each cell is able to rebuild its own specific albumin from the food, and does not take up the albuminous substances as they are present in the food without any change. We therefore distinguish two stages in the digestion and assimilation of food. One stage is merely analytical, a splitting stage. Here the different hydrolytic enzymes, such as lipases, amylase, saccharase, maltase, the proteases, develop their activity. In the following stage the reconstruction of cell-substance takes place, the synthesis of the organic principles of life. Modern chemistry has been fortunate enough to obtain even here remarkable results from experiments.

We should remember that hydrolytic processes

such as the decomposition of esters are reversible, and it only depends upon the conditions of the experiment where the position of the state of equilibrium is found : nearer to the ester or nearer to the products of decomposition. Analysis and synthesis are always connected. If a catalysing influence acts on such reactions, it must accelerate as well combination as decomposition. Else the process would not agree with the fundamental law of conservation of energy. We see that even enzymes which catalyse a hydrolytic decomposition must act even in the contrary direction, as a synthetical power. It was Van 't Hoff who first stated this postulate. A short time afterwards A. Croft Hill published his paper on the synthesis of malt sugar by means of maltase, which had hitherto been known only as a hydrolytic agent. When maltase was made to act on a very concentrated solution of grape sugar, it was noticed that a considerable quantity of a compound sugar was formed from glucose. It is true that later on it was shown that this sugar is not identical with maltose, but consists chiefly of isomaltose, a closely related sugar. Armstrong then showed that a real synthesis of maltose can be made by means of another enzyme, Emulsin, from grape sugar. Emulsin is further effective on the synthesis of the characteristic substance of bitter almonds, amygdalin. When amygdalin is treated with invertase, the cane-sugar-decomposing enzyme

of yeast, there are formed grape sugar and a compound which is a combination of glucose and the nitrile of amygdalic acid. Concentrated solutions of glucose and the nitrile-glucoside brought together with emulsin form in abundance amygdalin, the original glucosid of almonds, as O. Emmerling has shown. Undoubtedly synthetic effects were further observed, when lipase, the fat-decomposing enzyme, acted on a concentrated mixture of glycerine and fatty acids. Finally some synthetic effects are known from the enzyme which act on proteids. All these experiences render it very probable that the organic synthesis in cells is performed and regulated by enzymes, and we can no longer consider the formerly mysterious synthesis of organic compounds in life as a problem which is not accessible to chemical experimental investigation.

No less important prospects lie disclosed at present relative to the part of enzymes in the process of respiration. It was Lavoisier who clearly recognised that the respiration of animals was a process analogous to inorganic combustion. About 1800 Saussure, of Geneva, during his memorable investigations into plant nutrition discovered the respiration of plants. Since that time no doubt has existed that the fundamental laws of the process of respiration are the same in both the plant and the animal kingdom. It is true that in plants and in the lower animals one

characteristic is missing which most manifestly directs our attention to respiration as a process of combustion. It is the development of free caloric energy. But it is not difficult to show by means of suitable contrivances that each plant produces an abundant quantity of heat in respiration. We only have to keep germinating seeds in a Dewar-glass for several days to show that the temperature in the glass rises to 40 degrees and more. Careful isolation therefore is sufficient to demonstrate this production of heat. Physiological investigation taught that in both animals and plants the materials of combustion are essentially the same. Most frequently large quantities of fat, sugar, or carbohydrates disappear during the process of respiration. The striking feature in such chemical processes in life is that these substances are not used to produce new cell-substances, but in the first place to furnish free energy, which is used to maintain the life-processes.

The growth and the amount of respiration in a fungus or in germinating seeds show what great quantities of carbon dioxide are produced in a short time, and how much sugar is consumed in respiration. When we try to compare this vital decomposition of sugar with the sugar-decomposing processes which we apply in the laboratory, we shall find it astonishing what effects are produced in living cells without any high temperature, any strong chemical reagent or electric current. A

lump of sugar may be exposed to the air for years without showing more alteration than that it turns slightly yellow. Thus we come to the conclusion that organisms must possess special means which produce the rapid decomposition of respiration material.

The chemist Schoenbein, of Basel, was the first to show that enzyme-like substances take part in vital oxidation. He drew attention to the property of many plant tissues of turning a colourless emulsion of resin of guaiacum in water blue. He then showed that the effect on the guaiacum resin is also found in the filtered watery extract of the tissue, and that this oxidising effect cannot possibly be obtained if the extract be boiled beforehand. Later on numerous substances were found to be such oxidising ferments. All plant and animal cells contain such enzymes. But they act only on aromatic substances, as phenols and resin acids; on sugar or on fat they do not show any effect.

The explanation of this fact came from the discovery that pea-seeds, which are brought to germination without access of air, produce a large quantity of alcohol besides carbon dioxide. This process, which is found widely spread in plants which are kept without oxygen from the air, proved to be fully identical with the alcoholic fermentation of yeast. Even the enzyme which Buchner had found in yeast and had called zymase was stated to be present in higher plants. We

must consequently believe that the primary decomposition of sugar in plant respiration is closely related to alcoholic fermentation, if not identical with it. This is another type of respiration processes in the living cell.

The aromatic substances on which oxidising enzymes act seemed to have very little importance for cell-life until Palladin, of St. Petersburg, whilst working out experiments on plant respiration, came to a remarkable hypothesis. Most of the aromatic substances which are oxidised by the enzymes furnish dark colouring matters as products of oxidation. This can be shown when killed plants are kept in vapours of chloroform in an air-tight glass vessel. Quite commonly they turn a deep brown. Palladin supposes that such oxidation processes take place even in living cells, but the reduction of the colouring matters following immediately, no staining becomes visible. The aromatic substances therefore transfer the oxygen of the air by means of oxidases to other oxidable substances of the cell. This hypothesis explains quite satisfactorily the existence of enzymes which act only on aromatic substances, as well as the position of the latter substances in the metabolism of plants.

No small number of lower organisms are able to live without a supply of air or free oxygen. Pasteur discovered this important fact in yeast and bacteria. Yeast may live as well without

as with oxygen, and with some bacteria it is the same. For other microbes the presence of air is deleterious, as they soon die when brought in contact with a medium containing even only small quantities of oxygen. The possibility of life without oxygen can be shown by the following experiment. A flask is filled with a culture medium of sugar, pepton, and Liebig's extract of meat. This liquid is sterilised by boiling and infected with bacteria from teguments of bean-seeds. A quantity of soluble indigo is added to stain the liquid dark blue. Then the flask is well corked and allowed to remain for one or two days in the incubator at 25 to 30 degrees Celsius. After this time we are sure to see the liquid quite colourless, the soluble indigo being reduced by the anaerobic bacteria which develop rapidly and take the oxygen from the indigo. When the bottle is reopened and its contents poured slowly out into a dish, we see the liquid immediately colouring greenish, then light blue, and soon dark blue, as it was before. This change is brought about by the reabsorption of oxygen from the air. Such experiments show distinctly that bacteria can grow without more than minute traces of oxygen, and that under such conditions the bacteria are able to draw oxygen from its compounds by reduction. Different results that have been arrived at lead to the conclusion that enzymes also take part in this process

of reduction. These so-called *Reductases* seem to be widely spread in lower and in higher plants.

Finally, we have to report that enzymes take part in the formation of such products of metabolism as are no longer of any use for the organism. They are removed from it as excretions, or form in the tissue deposits which do not change. In animal life a great quantity of nitrogenous substances are eliminated from the organism, as urea and uric acid. It has been shown by several authors that enzymes participate when these excretion substances are formed. When the bacteria which cause putrefaction of meat are preparing their cell-substances from the proteins, a number of atom-groups from protein are eliminated as waste substances. Particularly when putrefaction is going on without sufficient access of air, many substances are formed which are responsible for the peculiar smell of putrid matter, and which are to be considered as bacterial excretions. Such are some compounds of sulphur, hydrogen sulphide itself, and methyl-mercaptan ; further, indol and scatol are substances which are very characteristic of putridity. No less must a series of phenols be mentioned as products of putrefaction. We have certain proofs for the view that all these substances take their origin from amino-acids, which are the primary products of the decomposition of proteids. By splitting of carbon dioxide and of ammonia the formation of

the substances mentioned above is easily explained, and it becomes more and more probable that enzyme reactions can cause these decompositions. In the case of some of these enzyme reactions we may be sure that they even occur in the cells of higher plants and animals, and are not confined to the lower organisms.

After our short review of the immensely extended territory of catalytic and enzymatic phenomena in the living cell, we cannot but confess that the importance of such processes is surprisingly great. The large number of different chemical reactions which take place in living protoplasm, and which we know from physiology to be the fundaments of chemical phenomena in life, is comparatively well understood at present on the basis of enzyme-chemistry.

It is true, there are some most important chemical processes in living cells which do not yet form part of catalytic chemistry. I may here mention the unique synthetical process in plants, the formation of sugar from the carbonic acid of the air by the chlorophyll bodies of green cells in sunlight. But any day may bring the revelation that even here catalytic phenomena are at work, and nothing at present excludes the supposition that enzyme effects take part also in these phenomena of plant life. If we suppress our feelings of satisfaction that Exact Science has been able to penetrate into these mysteries of life, there are

yet facts enough which show us how far we are from a thorough understanding of the life-process. The striking feature of the present state of biological science is that nothing that we discover sufficiently explains the intimate connection, the marvellous regulation of all processes in living substance. Up to our days the living cell has represented an unknown mechanism which reacts most accurately and corresponds to the present conditions and which possesses all abilities to preserve its structure and the species beyond the limits of life.

An exact knowledge of the chemistry and of the physics of the living substance will undoubtedly teach us far more of these hidden combinations than we know at present. I cannot but add that there is nothing to indicate that the phenomena of life are ruled by forces which are different from chemical and physical energies in inanimate Nature. The fundamental laws of energetics seem to dominate in all Nature. The two principles of the mechanic theory of heat govern everywhere. In animate Nature no case is known where the principle of Conservation of Energy is not followed. The more exactly physiological experimental work is carried out, the more care is taken to apply quantitative methods. Thus we have come into possession of a great number of data which invariably show that the transformation of energy obeys the same laws in

life as in inanimate matter. In inanimate Nature, further, we always meet with the important phenomenon that caloric energy can never be transferred from a colder body to a warmer one, unless other special processes render it possible. By itself heat can only be transferred from a warmer to a colder body. This law, well known in Lord Kelvin's utterance, that the energy present in the world has the tendency to dissipate, doubtless governs living matter as well as non-living. There is only one part of physiology which is not yet accessible to our methods and which we cannot prove to be ruled by the well-known laws of inanimate Nature. These are the psychological phenomena. At present we see no way to transfer physical and chemical methods to the phenomena of the psychical world.

CHAPTER IX

CHEMICAL ACTIONS ON PROTOPLASM AND ITS COUNTER-ACTIONS

HITHERTO we know living protoplasm as a complicated system of colloidal substances possessing a highly developed structure, and ruled by a great number of catalytic reactions. The complex of these reactions is able to maintain the cell-structure, to take up substances from outside the cell to digest them and to gain from them both energy and cell-substance for growth.

We have not yet completely treated of the mutual chemical interchange between the outer world and living cells. This influence consists in something more than in taking up food and giving off excretion substances. The whole life-process depends to an enormous extent upon external chemical influences. Minute traces of iron salts, scarcely to be ascertained by chemical analysis, possess the power of greatly accelerating growth and respiration. Life can be destroyed by other substances in quantities which are infinitely smaller than the mass of protoplasm which the deadly substance can injure. Such influences

may be called *Chemical Stimuli*. Their action is quite comparable to the action on living matter of physical stimuli, such as light, warmth, electricity and gravity.

It is quite a general rule that substances which produce poisonous effects on living cells when applied in a certain concentration, influence living cells quite differently when their concentration is more diluted. Then stimulating effects are regularly produced. Respiration and growth reach a higher degree than without application of the poison. For example, potato plants treated with copper sulphate show darker green leaves and more vigorous stems than normal plants. We see that poisonous action does not depend only on the chemical nature of substances, but also on the concentration of the substance. We should rather speak of poisonous effects than of poisonous substances. The explanation of the phenomena may be given by the principle of action and counter-action. The poison—for example, mercury chloride or carbolic acid—develops a retarding influence on some processes in living protoplasm. Protoplasm is by this action incited to react against the injuring influence. This is done by an acceleration of the chief processes of life—respiration, growth, and probably many others. So the toxic influence is paralysed. The successful counter-action against the poisonous agent cannot, however, take place when the toxic influence is too strong.

Then the latter prevails, and only the harmful consequences become visible.

The discovery of further interesting chemical stimuli was made in the course of the studies of the consequences of extirpation of certain organs, as the thyroid gland or the suprarenal bodies in animals. This procedure invariably causes fatal consequences for the organism. It is followed by serious disturbances of the normal metabolism and finally by death, so that there is no doubt that these glands perform important functions. But since the organs mentioned have no excretory duct, the substances produced by them must be transferred directly into the circulation of the blood. This internal secretion appears to be of the greatest importance. Seemingly very different substances are produced by these glands, not only proteids, but also aromatic carbon compounds have been stated to play a part in internal secretion. But all these substances exert stimulating and regulating effects on the organism. They are generally united under the name of *Hormones.* Even plants seem regularly to produce such substances. The swelling of the ovary after pollination is caused by certain soluble substances of the pollen. Very likely the formation of flowers, or of the sexual organs in lower plants, is connected with the occurrence of Hormones in the organism of plants.

Most remarkable chemical actions and counter-

actions are observed in living protoplasm when other cells and their products, not only an inorganic poison, are the injuring part. We may be reminded of the interesting phenomenon with which we became acquainted in the formation of anti-enzymes. In the animal which has had an enzyme solution injected into its veins, a substance is formed which is able to hinder the action of this but of no other enzyme. Such phenomena are widely spread and are most important for the study of chemical processes in cells. In studies on pathogenic bacteria it has been shown that many of them produce substances which are most poisonous even in the smallest quantity, but differ from other poisons by their albuminoid character and their instability when heated. By boiling they may be easily destroyed. Such poisons are formed only by living cells. We call them *Cytotoxins*. Such cytotoxins have become known not only from bacteria, but even from higher plants and animals. The fly-agaric and some of its relations, the seed of the castor oil plant and of Croton, as well as the seed of *Abrus precatorius*, the Jequirity plant, contain toxins of exceedingly strong action. Cytotoxins, further, are found in snakes, toads, the blood of the eel and some other fish. If we consider the characteristics of cytotoxins we feel very much reminded of the properties of enzymes. The resemblance increases when we learn that cytotoxins, quite in the same way as

the enzymes, cause the formation of specific anti-substances when brought into the veins. The formation of *Antitoxins* is quite analogous to the formation of anti-enzymes. Antitoxins have the specific effect of rendering the Cytotoxin, to which they correspond, inefficacious. This Antitoxin-phenomenon, as we know, plays an important part in the defence of animal and human organisms against the toxin-producing bacteria in infectious diseases.

The production of anti-bodies is a most remarkable feature in the mutual chemical influencing of living cells against alien living cells and their chemical products. Especially for pathology, the study of such phenomena is at present of the greatest importance. A whole new branch of biochemistry, called *Immunochemistry*, has been built up upon the basis of the general experiences mentioned above.

In our general review of the chemical phenomena in life we cannot but lightly touch on the facts which show how the living organism protects itself against the attacks of microbes. These facts are very interesting for us to illustrate how the protective substances and the aggressive substances of living cells may enter upon reactions. Cytotoxins, as well as enzymes, are typically colloidal substances, and so are antitoxins. When antitoxins neutralise the cytotoxins we could think that the cytotoxins would be destroyed. But it is

not so. If we heat the mixture of antitoxin and cytotoxin to nearly the temperature at which the latter is destroyed by heat, we can reach a point where the mixture again becomes toxic. We get the impression that the antitoxin in the compound has been sooner destroyed by heat than the cytotoxin, and the latter has again become free and effective. This most important experiment shows us that both anti-substances enter into a combination, analogous to that of chemical compounds. Since we know that both substances are colloids, we could suppose that colloid reactions are responsible for the phenomenon. Otherwise we could think that the reaction is to be considered a chemical combination of both substances. At present there are many difficulties in the way of giving a satisfactory explanation of the reaction. Arrhenius drew a most instructive parallel between the neutralisation of toxin and antitoxin, and the neutralisation of a moderately strong alkali, such as ammonia, with a weak acid, e.g. boric acid. Both processes, indeed, have a great resemblance. Ehrlich's ingenious hypothesis, well known as the so-called *Side Chain-Theory*, culminates in the supposition that the anti-substances represent highly compound molecules with many atom-groups, such as proteids possess. The neutralisation is done by binding two distinct groups. These groups may be destroyed by heat, and both substances again set free. Possibly the two theories

will one day be combined. The hypothesis of Arrhenius is more satisfactory for the scientific chemist. The theory of Ehrlich is founded upon a sound atomistic basis, and has proved of great heuristic value.

When toxin and antitoxin solutions are mixed, no change can be seen in the solution. With other anti-bodies it is quite different. It was found that the blood serum of animals which had been injected with bacteria of typhoid fever or *cholera asiatica* gave a strong precipitate with the limpid filtrate from cultures of the same bacteria. Even this effect is quite specific. Further, it was shown by a series of experiments that similar results are obtained by injection of different proteids into the venous system of animals. The blood serum is then able to precipitate the proteid which was injected, and exclusively this proteid, from its solutions. All these reactions were called *Precipitin Reactions.* They are in many respects most interesting. In the first place, they show that comparatively primitive protein-bodies cause the same anti-reaction as enzymes or cytotoxins. But only protein-bodies are known to give the reaction, no other organic compounds. When the proteid is decomposed by pepsin and hydrochloric acid the precipitin reaction cannot be obtained again. The simple amino-acids which are formed from protein in digestion do not give the precipitin reaction. But the reaction is also satisfactorily

obtained in albumoses and peptones, the most primitive protein-bodies. There is every hope of the possibility of soon explaining this reaction much more exactly than is at present possible.

But even now we see what complicated reactions can take place among proteids, and how easily precipitates are formed without seriously changing the original proteids. Most remarkable is the fact that the proteids of a species of plant or animal do not give any precipitin reaction with the blood serum of an animal treated with the proteid of the same plant or the same animal. Therefore the reaction can be used to distinguish whether a proteid is an alien one, or one belonging to a certain species. Experiments were made by Uhlenhuth on anthropoid apes, and on groups of lower apes. Anthropoid serum from animals which were treated with the blood of man does not give any precipitin reaction. But serum from other apes which were treated with the blood of man gives a distinct reaction. We see from this fact that the blood of anthropoids is not essentially different from that of man. The proteids are the same in both.

The result is that each species of organism has its own specific proteids. We understand now why the alien proteids which are taken in with the food have to be split up until they finally form amino-acids, so that the alien protein structure is quite annihilated. Then the cells

reconstruct the proteins according to the specific structure of protein which is characteristic of the particular species of organism. Further, we learn from the experiments on precipitin reactions that the morphological position of a species in the system is also physiologically founded. We may suppose that closely related species must also show chemical relations. The chemical mechanism of the precipitin reaction is not yet clear. We can think of the phenomenon mentioned in a foregoing chapter, that two colloids of contrary electric charge flake each other out. Since albuminous substances readily change the kind of electric charge, many opportunities would be given to cause such precipitate reactions. It has been shown without doubt that the precipitin is entirely consumed in the reaction. Therefore we cannot state that any resemblance exists with enzyme reactions. Living cells can even produce specific substances having the properties of proteids which have the power to agglutinate other cells or unicellular organisms such as bacteria. A similar effect is obtained by adding to a culture of typhoid bacteria in the test-tube some of the blood serum of an animal which had been previously treated with typhoid bacteria by intravenous injection. Flakes of bacteria are formed, between them the liquid becomes quite limpid, and the medium which had been turbid with bacteria shows itself later on quite clear, and

all the bacteria are found in the deposit. The substance responsible for this reaction, the so-called *Agglutination of Bacteria,* is destroyed by heat and has the properties of a protein-body. Substances of this kind we call *Agglutinins.* Even this reaction is a strictly specific one. The agglutinin produced by injection of a certain species of bacteria gives to the blood serum the specific agglutinating action on these bacteria. Agglutination effects occur even in other toxins. The toxin substance from the seeds of the castor oil plant strongly agglutinates the red blood cells, and so does the Jequirity toxin. There is no doubt that the agglutinin acts on certain substances in the bacteria-cells or other agglutinable cells. These substances are probably transformed into a gelatinous state, which is seen in the clinging together of the cells. The agglutinin is entirely consumed in this reaction. It may therefore rather be compared to a neutralisation than to an enzyme action.

The most successful study of the alterations which occur in the blood of animals, after intravenous injections of pathogenic bacteria and their products, showed far more substances formed which serve for the protection of the organism than we have here mentioned. But all these substances, such as opsonines, bacteriolysins, and, further, the bacterial substances, such as aggressines and others, which assist parasites

against their hosts, have hitherto not been of such general biological interest that we need treat of them.

This chapter had the purpose of showing that numerous chemical influences are exercised upon living protoplasm by the protein substances of other cells, and that such reactions have a markedly specific feature. The life process can be stimulated or retarded by these influences, production of certain substances can be provoked or hindered, and death can even be caused by such cell substances. We learned how far the substantial specificity goes in an organism. The structure in protoplasm is certainly not the only characteristic which is decisive for living substance. We have also continually to keep in mind the chemical nature of the substances in protoplasm.

Modern chemistry is not yet quite sufficiently advanced to clear up this most interesting complex of reactions between highly composed protein-bodies. It is still the question whether the reactions between toxins and their anti-bodies are really of ordinary chemical character, or whether they belong to the territory of colloidal reactions. Here is one of the most suggestive problems of modern Biology. There is no doubt that enormous progress will come from further study of Immunochemistry.

CHAPTER X

CHEMICAL ADAPTATION AND INHERITANCE

OUR review of the chemical phenomena in life would not be complete unless we had a last glance at the chemical phenomena of variation, adaptation and inheritance in living beings. The investigation of these phenomena lies at present so much within the territory of morphology that one scarcely thinks of the importance of chemical work in this department of biological science. Chemical methods, however, are here of particularly great interest. Morphology, being a comparative science, draws attention only to the *results* of variation and adaptation. Chemistry has to show the *whole course* of phenomena, not only the results, and it has to consider the influence of time on phenomena, to determine the minima and maxima in the course of reactions, and to introduce the *Time Factor* into all these investigations. In chemistry, therefore, variation can be observed in the course of phenomena as well as in the final results. Since alterations and variations in the course of physiological actions can generally be traced back to the influences of

certain factors, chemical methods open up an immensely wide outlook.

At present chemical investigations into variation and inheritance unfortunately show so many gaps that our report cannot be but a provisional one, and it must rather contain suggestion for fresh experimental work than material already worked out.

The kinds of variations which morphologists distinguish as *Fluctuating Variation* and *Mutation* are exactly repeated in the chemical properties of living organisms. The Law of Fluctuating Variation discovered by Quetelet is expressed by the statement that the average values are the most frequently recurring ones. The individuals showing a certain characteristic more or less marked, are rarer, the greater the divergence from the average value or average size of the characteristic. This law, which can so regularly be shown by measuring the length, weight or volume of an organ of plants or animals in a great number of individuals, supplies exact returns in chemical variations. De Vries gives a report of the result of an examination of 40,000 sugar beets with regard to their content of cane sugar. From the curve given by De Vries we immediately recognise the fundamental law. The average quantity of about 16 per cent of sugar was found in nearly 7000 beetroots; 12 per cent sugar in only 340 roots, 19 per cent in only 5. It is true that such

research work has not been carried out very often, but the few experiments which have already been made render it most probable that Quetelet's law holds for chemical properties as well as for morphological characteristics. It would be comparatively easy to examine the amount of acid contained in leaves, the amount of starch or of protein which is contained in one individual in a great number of cases in order to confirm the results mentioned above. No research work at all has been done to determine the velocity of chemical processes or reactions in a great number of single individuals. Data without any difficulty could be worked out on the velocity at which starch or protein disappear from germinating seeds or on the intensity of respiration in many individuals which live under exactly the same conditions. It is difficult to say what results would be thus obtained. In any case such research work is highly desirable.

The second kind of variation takes place suddenly, eruption-like, and culminates in the production in single individuals of quite different characteristics which are markedly inheritable. Since De Vries' famous book on these phenomena, we call such variations *Mutations*. Chemical mutations are widely spread and well known. In horticulture and agriculture many new mutations which were kept on account of their valuable chemical properties have in the course of time been isolated. Fruits, containing an extraordinary

quantity of sugar, or of peculiar aroma and taste, or corn containing a considerable quantity of starch, are examples of such sudden chemical variation. Doubtless to these chemical mutations may be assigned all the results which were obtained in morphological mutations. But even here it is unknown whether mutations occur in the velocity of reactions or vital processes in single individuals, out of a great number of plants or animals, and whether such variations are well fixed and inheritable. Well worthy of exact examination would be, further, the question how chemical variation works in hybrids. It is well known that the progeny obtained by crossing two species of animals or plants, in many cases follow the rule that only half the progeny remain of hybrid character, but the other half return to the parental types. This law is the famous *Mendel's Law*. Up to our days we do not know whether chemical characteristics may "mendel" too. It is likely to be so in many cases, and could without difficulty be confirmed at least in a number of experiments. If chemical Mendelism could be discovered, it would be of great interest, because it lies in the nature of Mendelian characteristics that they are based on qualities of the nuclei of the sexual cells.

A further type of variation is known as *Atavism*. In the formation of a certain characteristic some individuals of the progeny return to the stage

of this characteristic in the ancestors. There is no doubt that chemical atavism will frequently be found in connection with morphological atavism. We need only think of the reappearing characteristic of the uncultivated ancestors of our fruit trees. But it is not yet known whether such chemical atavisms can reappear without being accompanied by morphological atavism.

Finally, we have to turn our attention to the variations which are caused by external influences. Botanists well know that the size and thickness of leaves depend upon the intensity of the sunlight in which they have grown. Especially the intensity of light, but also the degree of moisture in the air, gravity, mechanical and chemical influences cause very remarkable alterations in the morphological characteristics of plants. At the same time chemical alterations must take place, and we see at last from all the research work which has been carried out in that domain, that the variation is not merely a morphological one, but is also chemical. One must feel it to be a great gap in biological work that chemical properties in their dependence on the physical and chemical influences of their surroundings have not yet been investigated for themselves alone. But a number of facts show even now that chemical variation depends on the influence of environment, and that it shows a similar purposive tendency towards adaptation to the environment, as is known in

morphological characteristics and variations. The oil-seeds of the plants of the flora of our country always contain fat which is liquid at temperatures of above 10 to 20 degrees Celsius, and becomes solid at a few degrees above zero. Tropical plants very frequently contain fat which melts only at a temperature above 30 degrees, and is solid at an average European temperature. This difference is likely to be connected with the temperature in which the plants live. Another phenomenon of the same kind is the rule in the production of enzymes. In moulds no amylolytic enzyme is produced unless these fungi grow on culture medium containing starch, and the common grey-green mould *Penicillium glaucum* produces an enzyme which destroys wood-substance, when it grows upon wood, but never when it grows on other substrata. For the formative action of chemical and physical influences on the morphological qualities of organisms the term *Morphoses* has been introduced. In an analogous manner we can name the chemical alterations provoked by these influences in plants and animals *Chemoses*. Morphoses are to be considered as reactions of the living organism to external stimuli. They belong to the physiology of stimuli, and we cannot but assume that they differ from tropisms and other primitive forms of reactions only in their complexity. Chemoses must be considered as reactions of the living organism in the same way, and all

that is known about morphological reactions must be assigned to these reaction-phenomena.

Biologists are nowadays inclined to explain the phenomena of adaptation in plants and animals by the supposition that the hereditary adapted forms took their origin from transitory morphoses, which often do not last longer than the time during which the external stimulus is acting on the organism. In such a way may for instance be understood the origin of dorsiventrality in plants. A branch of ivy develops its rootlets only on the shade-side, and turns its leaves all to the sun-side. If we turn the branch by 180 degrees and fix it in this new position, it changes its morphological properties entirely, corresponding to the new conditions. The old rootlets shrink and fall, but new climbing roots are formed on the side which is now turned away from the light. The dorsiventrality is, as we see, not fixed. A branch of a pine tree when turned by 180 degrees behaves quite differently. The old part does not change its character, and in spite of the unnatural position the leaves remain without any reaction. But when in the following spring the branch continues its growth, the new part of the branch corresponds in its formation exactly to the new position. We see that a reaction could not be carried out in the adult part of the branch, but the characteristics of this part were not transferred to the new part. The latter behaves according to its real life con-

ditions. Again, the thallus of a liverwort, such as Marchantia, shows differences. If a young gemma of the moss is exposed to light in a certain position, the lighted side is destined to be the upper surface for ever, and the opposite side to be for ever the root-producing under surface. Nothing can change this. Such a case corresponds to adaptation, it is strictly hereditary, and must be called a purposive reaction, because the proper tissues develop on both the light-side and the under surface.

We may be sure that thorough investigation of chemical phenomena in life will certainly disclose analogies. Most probably the self-steerage in the production of enzymes belongs to a series of such phenomena. On the other hand, the above-mentioned formation in tropical plants of fats of a high melting-point may be called a perfect chemical adaptation.

Phenomena of inheritance of chemical properties are as well known as those of hereditary morphological properties. We know only how far morphological and chemical properties are inheritable together, and how far chemical properties separately are hereditary. Nevertheless, examples of chemical varieties show that sometimes only one chemical characteristic varies, and no other. The bitter almond shows no difference from the sweet variety of almond, but by the presence of amygdalin. This case of heredity depends upon fecundation processes,

since the progeny of bitter or sweet almonds, respectively, invariably show their peculiar characteristic. Consequently the characteristic of producing amygdalin depends on the nuclei of the sexual cells. Generally, we speak of heredity only when sexual processes are involved, and the properties of one generation are transferred to the following generations. In plants, however, it is possible to take the conception of heredity in a wider sense. *Sensu stricto* a sexual cell with its properties is a part of the parental organism which is separated from the latter and is beginning an independent life. For heredity I think we must not lay too much stress upon this circumstance, and it does not matter whether the transferring of parental properties takes place among cells which remain connected or not. When in a growing branch the young part acquires its properties from the adult part, this process is done by cell cleavage, each cell transferring its characteristics to its daughter-cells. We may consequently here also speak of phenomena of inheritance, and we shall distinguish them as *Asexual Inheritance.* The term Inheritance implies that the transferring of characteristics takes place continually from generation to generation. But it is not necessary for the characteristics to be apparent. Hybrids often do not show their characteristics in an intermediate form between the parental forms, but entirely resemble in a certain respect one of their parents.

Mendel showed that in the second generation the hidden characteristic of the other parent becomes manifest in 25 per cent of the descendants. So it must have been latent in the first generation. Such cases of heredity we call *Discontinuous Heredity*, continual manifestation of characteristics *Continuous Heredity*.

Heredity is far from being an absolutely sharp and marked conception. Phenomena of typical sexual inheritance are connected by an innumerable range of intermediate stages with the phenomena which we call typically transitory inductions. One could even think that Inheritance represents only the limit of longeval induction, of which we cannot recognise the end, because the duration of our time of observation is too short. If we could follow up millions of generations, if we could have the age of an eternal being, we might find the phenomena of variation more striking than the phenomena of inheritance. The best materials with which it is possible to observe a great number of generations in a few weeks are microbes and bacteria. There is one case known which illustrates the conception of inheritance most instructively. The *Bacillus prodigiosus* is a microbe which, under normal conditions, is very noteworthy because of its production of a scarlet colouring matter. When this bacterium is cultivated at a temperature of 30 to 35 degrees it gradually loses its colour. The interesting fact

is now that the property of being colourless remains when the microbe is again cultivated at the ordinary temperature of 18 degrees. One would feel inclined to suppose that it had lost its property of producing the red pigment by the influence of heat. The loss is undoubtedly hereditary, for many generations are formed under normal temperature conditions which are absolutely without any red hue. But after a certain number of generations, which may be many thousands, the red hue returns, and the bacterium regains its former appearance. Such phenomena seem to be not very rare. If we were beings of quite short duration of life, we would perhaps believe that the loss of red pigment in these bacteria was real inheritance. Since we can prove that after a great number of generations the former property returns, we call that *Pseudo-Inheritance.* But we must bear in mind that there is no sharp distinction between pseudo-inheritance and real inheritance. The latter can only be considered as a pseudo-inheritance which lasts for an infinitely great number of generations. Chemical phenomena in this territory will certainly be discovered, and perhaps will contribute much towards making these difficult questions clearer.

Phylogenetic investigations still contain many more interesting chemical questions than we could touch on in our short discussion. Well worth consideration is the question whether the so-called

Biogenetical Law of Haeckel extends to chemical phenomena. We know that the embryos of higher animals show considerable morphological resemblances to lower animals, and so it is in plants. The first stages of development in mosses resemble algæ, the first development of ferns reminds us very strongly of liverworts. These facts are so general that they have been summarised in the rule: *That the development of the individual organism or the ontogeny represents a short recapitulation of the phylogeny.* This law is hitherto only based upon morphological facts. Since morphological phenomena are always accompanied by chemical analogies, we may suppose that the law of Biogenetics can be applied also to chemical phenomena in life. Many reasons can be produced to support this idea. The primitive groups of higher plants, such as Mosses and Ferns, and Gymnosperms, do not contain by far as many different substances as the Phanerogams. All the numerous glycosides, most alkaloids, and the bitter principles occur in the phanerogamic groups. The lowest plants of the classes Algæ and Fungi in general contain only the widespread organic compounds, such as fats, carbohydrates, or proteids. The Lichens, a highly developed symbiotic group of Fungi, alone contain a greater number of specific organic compounds belonging to the class of benzene-derivatives. The lowest Algæ and Fungi as well as the Bacteria

have essentially the chemical composition of protoplasm. In Ontology we see that the young tissues of higher plants do not yet contain the different chemical compounds which are found in the adult plants. Even here the chemical composition of the youngest cells is essentially that of protoplasm.

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p. 51. Etching of marble by plant roots,
This probably explains the curious markings on the surface of the Lower Carboniferous (Carboniferous) of Long buried (Carboniferous).